

## Microbiological characterization of the millet-based (*Pennisetum glaucum*) *Ablo* and sorghum-based (*Sorghum bicolor*) *Ablo* produced in Benin

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### Abstract

*Ablo* is wet bread, slightly salty and sweet, steamed and sold in the form of pellets. The objective of the study was to assess the microbiological quality of millet-based *Ablo* and sorghum-based *Ablo*. The methodology adopted was to perform production tests followed by analyses in the laboratory. The results showed that the lactic acid bacteria, yeasts and moulds were the dominant micro flora of the millet-based *Ablo* and sorghum-based *Ablo*. Furthermore, analysis showed that the millet-based *Ablo* and the sorghum-based *Ablo* doesn't contain either total coli forms, coli forms thermo tolerant or consequently *Escherichia coli*.

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## Introduction

In Benin, corn, main cereal in the food system, investigated a multitude of transformations leading to more than 40 different products (Nago, 1989). Among these many products, Fig. the *Ablo*, a dough drying corn, slightly salty and sweet of origin *Mina* prepared from the mawe, sometimes added to wheat flour and of various ingredients (yeast, salt, sugar). Traditionally consumed in Benin, Togo and Ghana, he is poised to win the West-African (Akakpo, 2013). However, the product of our days, is subject to significant changes in its preparation, mainly regarding the raw (maize, rice and wheat). The partial introduction or the substitution of rice in the preparation of the *Ablo* is a fairly recent but increasingly observed practice in urban areas. These types of *Ablo* meet consumer's acceptability and allow satisfying the desire of the customer in relation to its requirement related to its new styles and eating behaviours (Akakpo, 2013).

The expansion of cities and the distance between the home and the workplace promote the increase of consumption outside the home, in the small restaurants and more particularly with the street vendors (Banon, 2012). Today, street food receives a more numerous and diverse clientele including all socio-professional categories (Bokossa *et al.*, 2013). It is an important area of activities involving large sums of money and source of jobs in a large proportion of the population, including women and families (Aholou-yeyi, 2007). In urban areas, low productivity of these traditional crafts enterprises and the tedium of some unit operations induce profound changes at the level of processes. A thorough knowledge of the endogenous technologies for food processing and their variability would contribute to their optimization.

This study focused on two new types of *Ablo* namely: the sorghum-based *Ablo* and millet-based *Ablo*. The presented information is gotten following experiences of consistent production of analyses in the laboratory.

The study permitted us to master the microbiological quality of the two new types of *Ablo*. The study aimsto assess the microbiological quality of the sorghum-based *Ablo*and millet-based *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 material of analysis consists of classical material used for microbiological handling.

#### *Other materials*

The material used for the different manufacturing was constituted of ingredients (sugar, salt) and usual equipment of for the production of *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.

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## 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. We realized samples at the end of the fermentation and after cooking. The various microbiological analyses on these samples.

### **Methods of analysis**

Microbiological analyses consisted of counting the total flora, of yeast and moulds, lactic acid bacteria, total and thermo tolerant coli forms by crops on synthetic media. 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.

### *Count of total flora (ISO 4833, 2003)*

From each prepared decimal dilution, 1ml 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 were added, and then all was perfectly homogenized. After complete solidification, plates were incubated at 30°C for 48 to 72 h.

### *Enumeration of yeasts and moulds (NF ISO 21527-2, 2008)*

The medium that was used for their research was the word Dextrose Agar (Oxoid CM 0041) to chloramphenicol (0.05 g/L). Previously prepared and sterilized, 10 to 15 ml of Word 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 incubation plates at 25°C for 3 to 5 days.

### *Enumeration of lactic acid bacteria (NF ISO 15214 (V 08-030), 1998)*

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 15 ml 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 48h.

### *Enumeration of total coliforms (NF ISO 4832 (V 08-015), 2006)*

One (1) ml of each prepared dilution was placed in sterile Petri dish boxes. There was paid then the violet Red Bile Agar (VRBA Oxoid CM 0107). After solidification, a second layer was conducted. Boxes of Petri dishes were incubated at 37°C for 24 h.

### *Enumeration of thermo tolerant coli forms (NF V08-060, 2009)*

One (1) ml of each prepared dilution was placed in sterile Petri dish boxes. There was paid then the violet Red Bile Agar (VRBA Oxoid CM 0107).

After solidification, a second layer was conducted. Boxes of Petri dishes were incubated at 44°C for 24-48h.

#### 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.

#### 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

Results of microbiological analyses of the fermented dough of the two types *Ablo* have been indicated in table I and table II. Microbiological analysis has revealed the absence of germs such as total coli forms and thermo tolerant coli forms. Load the total flora of lactic acid bacteria, yeasts and moulds was higher in the fermented pulp (millet and sorghum) uncooked in the two types of *Ablo* after cooking.

Thus, the total flora spent of  $7.44 \pm 0.12$  to  $2.95 \pm 0.17 \text{ Log}_{10} \text{ cfu/g}$ , lactic acid bacteria of  $6.073 \pm 0.28$  to  $4.80 \pm 0.64 \text{ Log}_{10} \text{ cfu/g}$  and the yeast and moulds of  $7.50 \pm 0.14$  to  $4.22 \pm 0.45 \text{ Log}_{10} \text{ cfu/g}$  (Table I).

In the fermented pulp of uncooked sorghum and the sorghum-based *Ablo*, the load of the total flora, of lactic acid bacteria and yeasts and moulds ranged respectively from  $6.42 \pm 0.23$  at  $2.03 \pm 0.13 \text{ Log}_{10} \text{ cfu/g}$ , of  $8.04 \pm 0.66$  to  $4.99 \pm 0.73 \text{ Log}_{10} \text{ cfu/g}$  and of  $8.53 \pm 0.32$  to  $5.42 \pm 0.78 \text{ Log}_{10} \text{ cfu/g}$  (Table II).

**Table I.** Microbiological characteristics of millet fermented dough and that of millet-based *Ablo*.

	Product average values of the desired microorganisms ( $\text{Log}_{10} \text{ cfu/g}$ )				
	Total flora	Yeasts and moulds	Lacticacid bacteria	Total coliforms	Thermotolerant coliforms
Millet fermented dough	$7.44 \pm 0.12^a$	$7.5 \pm 0.14^a$	$6.73 \pm 0.28^a$	Absent <sup>a</sup>	Absent <sup>a</sup>
Millet-based <i>Ablo</i>	$2.95 \pm 0.17^b$	$4.22 \pm 0.45^b$	$4.80 \pm 0.64^b$	Absent <sup>a</sup>	Absent <sup>a</sup>

Mean values with the same letter in the same column are not significantly different at the 5% level. Data represents in table is mean of three replications.  $\pm$  Standard deviation.

**Table II.** Microbiological characteristics of the fermented dough of sorghum and the sorghum-based *Ablo*.

	Product average values of the desired microorganisms ( $\text{Log}_{10} \text{ cfu/g}$ )				
	Total flora	Yeasts and moulds	Lacticacid bacteria	Total coliforms	Thermotolerant coliform
Sorghum fermented dough	$6.42 \pm 0.23^a$	$8.53 \pm 0.32^a$	$8.04 \pm 0.66^a$	Absent <sup>a</sup>	Absent <sup>a</sup>
Sorghum-based <i>Ablo</i>	$2.03 \pm 0.13^b$	$5.42 \pm 0.78^b$	$4.99 \pm 0.73^b$	Absent <sup>a</sup>	Absent <sup>a</sup>

Mean values with the same letter in the same column are not significantly different at the 5% level. Data represents in table is mean of three replications.  $\pm$  Standard deviation.

## Discussion

The most common microorganisms in fermented doughs, on the *millet-based Ablo* and sorghum-based *Ablo* are yeasts, mould and lactic acid bacteria.

The same comments were made by Ahokpe (2005), by Aholou-yeyi (2007), by Banon (2012) and by Bokossa *et al.* (2013) who said that the dominant microflora of the *Ablo* consists of lactic acid bacteria and yeasts and moulds.

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These microorganisms come from the commercial yeast added but also from the contamination of flour during milling. The results are consistent with those obtained by Ahokpe (2005) and Aholou-yeyi (2007) on the *Ablo* corn who pointed out that the micro flora present in corn grain, the equipment used and in the mawe or the flour contaminated during grind play a role in the fermentation.

The Two types of *Ablo* developed contain neither total coliform, fecal coliform nor *Escherichia coli*. These results mean that good hygiene practices are met during production and indicate that the manufactured *Ablo* present no danger to human health. The same comments were made by Tchekessi *et al.*, 2013, Banon (2012), Tchekessi (2012), Clabots (2007), Leclerc *et al.* (1977) reported that the absence of enterobacteria is a good indicator of the level of acceptable hygiene in the production of food. They said that the enterobacteria are witnesses of contamination after heat treatment and that their presence in large numbers indicates a failing hygiene during production but also an ability of the product to promote their development.

The total flora found in the two types of *Ablo* is due to contamination after cooking. These results are consistent with those of Turpin (2010) and Tchekessi (2015) which attested that the total flora encountered in the food after cooking comes from the environment. The reduction of the microbial load on products after cooking is explained by the fact that microorganisms are partly destroyed by heat. Cooking at the same time plays the role of pasteurization. Alais (1984) and Vignola (2002) show that the destruction of the microorganisms is function of two parameters such as the temperature and the duration of the heat treatment. Similarly, Leclerc *et al.* (1977), Akapko (2013), Agro (2013) and Bokossa *et al.* (2016) have mounted that a heat treatment for a few seconds at 72°C is sufficient to destroy the vegetative forms of microorganisms in food products.

## Conclusion

Yeasts, moulds and lactic acid bacteria are predominant germs of millet-based *Ablo* and sorghum-based *Ablo*. The absence of total coli forms and thermo tolerant coli forms is a good indicator of the level of acceptable hygiene during the production of the two types of *Ablo*. We can say that the millet-based *Ablo* and sorghum-based *Ablo* are healthy foods in microbiological terms and therefore present no danger to human health.

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