Effect of the fermentation on the microbial population occurring during the processing of zoom-koom, a traditional beverage in Burkina Faso

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Zoom-koom is a traditional fermented beverage from Burkina Faso based on cereals such as millet or sorghum. Samples were collected from two local production sites of microenterprises (Zogona and Dassasgho). Microorganisms dynamic during the production of zoom-koom were enumerated using pour plate methods. The titratable acidity, pH and temperature of fermentation were determined using respectively titrimetric and electrochemical methods. The results showed a decrease in pH and an increase in acidity during soaking and fermentation step. While the enterobacteria and yeasts counts decreased (p<0.05), lactic acid bacteria (LAB) counts increased (p<0.05). On average, the pH decreased from 5.7 to 4.1; the lactic acid concentration ranged from 0.45 to 0.71 (lactic acid g/100 g) and the LAB ranged from 2.2×10\textsuperscript{8} to 5.6×10\textsuperscript{8} CFU/g for the millet dough. For the red sorghum dough the pH decreased from 6.2 to 4.2; the lactic acid concentration increased from 0.15 to 0.49 (lactic acid g/100 g) and LAB ranged from 8.9×10\textsuperscript{6} to 5×10\textsuperscript{10} CFU/g. The ambient temperature and nature of the grains had an impact on the fermentation process. Unfermented red sorghum zoom-koom had the lowest load of yeast and enterobacteria than unfermented millet zoom-koom. Short rods in pair or short chains (3 or 4 rods) bacteria are the main microorganisms responsible of the fermentation process.

**Key words:** Sorghum, millet, fermentation, zoom-koom, lactic acid bacteria (LAB).

INTRODUCTION

Spontaneous or natural fermentation has been used in Africa for centuries to preserve and improve the nutritional status of foods. Fermented sorghum or millet-based foods, alcoholic and non-alcoholic drinks or beverages are prepared in many African countries for human consumption (Odunfa et al., 1996; Usha et al., 1996; Muyanja et al., 2002). The desired properties of these beverages are their nutritional value, taste, mouth file and

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fruity aroma. Generally, drinks are not taken as food but in Africa, cereals-based beverages are considered as foods because of their nutritional value and their contribution to the diet of people (Sawadogo-Lingani et al., 2008). These beverages are highly appreciated by consumers, and play an important role in the culture of African people. Often attached to the traditions of hospitality and conviviality, these beverages are part of the etiquette of most families and serve to seal the relationships between individuals (Aka, 2008). Among these beverages, we have tchapalo or dolo and zoom-koom in Burkina Faso, pito in Ghana, doro in Zimbabwe, bouza in Egypt, kunun-zaki in Nigeria and mougoudji in Mali (Olasupo et al., 1997; Djé et al., 2008; Sawadogo-Lingani, 2010).

The zoom-koom is a non-alcoholic beverage, prepared from millet and rarely from sorghum, and much appreciated by consumers in Burkina Faso. It is produced from shelled cereal or whole grains. The grain of sorghum is distinguished from the grain of millet by its richness in polyphenols (tannins). Cereals aleuronic cells are rich in mineral salts, B-complex vitamins and lipids; they contain some hydrolyzing enzymes (Kiemtoré, 2005; FAO, 2012), but the husking and the blasting eliminate a good part. The rate of dietary fiber is variable (2 to more than 30%) and it depends on the size of the grain (Kiemtoré, 2005; FAO, 2012). Fibers play an important physiological role in allowing the normal progression of the alimentary bowl in the digestive tract and promoting certain metabolisms (cholesterol, triglycerides). The husking removes a good part of the bran, therefore fibers. The advantage of consuming a zoom-koom based on cereal not cracked is its richness in nutrients and dietary fibers. To get this drink, the grains of millet or sorghum are soaked overnight and soaked grains are washed and mixed with ginger and mint. The blend is grinded into a dough, diluted with water, and then filtered using a fabric to obtain zoom-koom, in which sugar and tamarind juice are added to give a sweet and sour taste (Soma, 2014).

However, according to Barro et al. (2007), zoom-koom is the street food which contains more count of thermostolerants and coliforms bacteria. To improve the microbiological quality of this beverage, a fermentation step of the dough is necessary. In fact, Soma (2014) had shown throughout a control fermentation of millet zoom-koom with Lactobacillus fermentum strain used as a starter, that the lactic fermentation allowed the reduction of enterobacteria counts and kept safe the final product. More ever, many works showed that the organic acids produced during the fermentation of pito in Ghana, tchapalo in Ivory Coast and ben-saagal in Burkina Faso, helped to obtain a better microbial stability of the product (Tou et al., 2006; Djé et al., 2008). Several investigations have shown the involvement of LAB in African traditional fermented cereal-based foods and beverages, including Lactobacillus, Leuconostoc, Lactococcus, Pediococcus and Weissella species (Olasupo et al., 1997; Hayford et al., 1999; Lei and Jakobsen, 2004). The microbiota of many African traditional fermented cereal products have been investigated, e.g. maize based products like kenkey (Halm et al., 1993; Olasupo et al., 1997; Hayford et al., 1999), mawè (Hounhouigan et al., 1993a,b, 1994) and ogi (Johansson et al., 1995; Olasupo et al., 1997), sorghum based product like kisra (Hamad et al., 1992) and dolo (Sawadogo-Lingani et al., 2007), millet based products like kunu-zaki (Olasupo et al., 1997) and ben-saalgo (Tou et al., 2006). However, only a few studies have been done to date on zoom-koom (Barro et al., 2007; Soma, 2014) and its fermentation process and no information is available on the kinds of micro-organisms involved in the spontaneous fermentation of the dough.

This study is to evaluate the effect of spontaneous fermentation on microbial population during the production of a zoom-koom based on fermented and unfermented whole millet (Pennisetum glaucum) and red sorghum (Sorghum bicolor L. Moench) dough.

MATERIALS AND METHODS

Monitoring of the zoom-koom processing and sampling

The processes of zoom-koom based on fermented and unfermented dough of whole millet (P. glaucum) or red sorghum (S. bicolor L. Moench) were followed in two production sites at Ouagadougou (Burkina Faso): one site is located in the district of Zogona and the other one is located in the district of Dassasgho. The diagrams of production process were established and the different steps of sampling were identified. The main steps and components were illustrated in diagram of the processes of both fermented and unfermented fresh zoom-koom by local producers in Zogona and Dassasgho at household level (Figure 1).

From this diagram, the different steps of sampling were indicated such as: the soaking of millet or red sorghum grains (start and end), the fermentation of millet or red sorghum dough (start and end) and their corresponding zoom-koom samples (without sugar and with sugar), the unfermented dough of millet or red sorghum and their corresponding zoom-koom samples (without sugar and with sugar after acidifying with tamarind juice). As shown in Figure 1, the production process of zoom-koom includes the following main steps:

(1) Soaking: After washing, the millet or sorghum grains were weighed and soaked in a quantity of water equal to twice of the mass of millet or sorghum grains (2:1, w/w). The soaking time was set at 16 h (soak time observed in micro-workshops).

(2) Grinding: The flavored and aromatization ingredients were added to the soaked grains (millet or sorghum) at the rate of 3 g/100 g for the mint and 6 g/100 g for the ginger, before the wet grinding.

(3) Fermentation: The dough is left to stand for fermentation for 10 h (average time observed in the field). The fermentation was realized at ambient temperature (33 to 42°C).

(4) Filtration: A quantity of water equal to 3 times the mass of the wet dough was added to the dough. The suspended is poured onto a muslin (mesh ≤ 0.5 mm) to get a zoom-koom without sugar.

(5) Mixture: After filtration, a sugar solution was added to filtrate to give the fresh zoom-koom with sugar (Figure 2). For the case of the unfermented fresh zoom-koom, a tamarind juice was added to filtrate in order to give the zoom-koom, a sour taste. The tamarind juice is not added to fermented zoom-koom because this product is
already acid. To make the sugar solution, a quantity of sugar is weighed and dissolved in a quantity of water equal to twice the mass of sugar (2:1, w/w).

Prior to sampling, all materials used for sampling (glass bottle) were sterilized at 121°C for 15 min. For each sampling, 200 g or 200 ml of sample were taken in sterile screw-cap bottles at the different sites.

Figure 1. Flow diagram of fresh zoom-koom to the producers at Zogona and Dassasgho sites in Ouagadougou (Burkina Faso). *Steps of sampling.

Figure 2. Zoom-koom of millet (A) and Zoom-koom of red sorghum (B).
steps of zoom-koom processes at both production sites. The samples were collected during three successive productions and transported to the laboratory in an icebox containing the ice. All the samples were preserved at 4°C before analysis which was carried out in triplicate, within the 24 h of the sampling. The temperature of the place from where the samples were collected was around 40°C for the production realized in Zogona site, (Months of April and May) and around 33°C for the production realized in Dassasgho site (Months of July and August).

**Enumeration of microorganisms**

For all samples, 10 g were homogenized in 90 ml sterile diluent (0.1% peptone, 0.8% NaCl, pH 7.0 ± 0.2) in a stomacher bag and homogenized in a stomacher (stomacher 400 lab blender, England) for 30 s at normal speed. For the solid raw material, the product was soaked for 30 min in the diluent at the laboratory temperature (25°C) before homogenization in the stomacher for 2 min at normal speed. From appropriate ten-fold dilutions, total mesophilic cells count were enumerated by pour plate on Plate Count Agar (Liofilchem, Spain) incubated at 30°C for 72 h (ISO 4833, 2003). Yeasts were enumerated by pour plate on Dextrose Chloramphenicol Agar (Liofilchem, Spain), pH 6.6 ± 0.2, and incubated at 30°C for 3 to 5 days according to ISO 7954 (1998). LAB were enumerated on modified Man, Rogosa and Sharpe (mMRS: MRS-IM agar + maltose) agar (Liofilchem, Spain), incubated anaerobically in an anaerobic jar with anaerocult A at 37°C, for 72 to 96 h according to ISO 15214 (1998). Enterobacteria were enumerated on Violet Red Bile Glucose (VRBG) agar (Liofilchem, Spain), incubated at 37°C for 24 h according to ISO 7402 (1993). Dishes containing 15 to 150 colonies were retained for the counting. The results were given as CFU/g of sample.

**Isolation and preliminary characterization of LAB isolates**

For LAB, the colonies from the highest dilution on mMRS were picked and further subcultured by streaking on mMRS agar anaerobically and growing in broth media (MRS broth) until pure cultures were obtained. A total of 366 isolates of presumed LAB were isolated. The isolates were first characterized based on colony and cell morphology using microscope (Olympus optical, BX 40F-3, Japan). Gram reaction was carried out by the KOH (3%) method (Gregersen, 1978), catalase production was determined by adding to a colony on a glass slide a drop of H2O2 solution (30%). Oxidase reaction was carried out by using oxidase disc. LAB isolates were subcultured and stored at -80°C in MRS broth with 30% (v/v) glycerol (87% v/v) for their identification.

**Physico-chemical analyses**

The pH of the samples was measured with an electronic pH meter (Model HI 8520; Hanna Instrument, Singapore). For solid samples, 10 g of product were mixed with 20 ml of distilled water prior to pH measurement. For liquid samples, the pH was measured directly (Sawadogo-Lingani et al., 2007). The temperature at the beginning and at the end of fermentation was measured with a thermometer (Rweger V104, Swiss). For titratable acidity determination, 5 g or 5 ml of sample suspended in ethanol (90%) was centrifuged for 5 min at 3500 g. Of the supernatant, 10 ml was transferred to a 50-ml measuring flask and filled up to 50 ml with distilled water. After mixing, 10 ml of the diluted sample was tittered with NaOH 0.1 N using 1% phenolphthalain as indicator (Sawadogo-Lingani et al., 2007). The titratable acidity (as g lactic acid per 100 ml or g of sample) was calculated according to Amoa-Awua et al. (1996).

**Statistical analyses of the data**

All the data were submitted to analysis of variance (ANOVA) and a principal component analysis (PCA) with the statistical software XLSTAT-Pro 7.5.2 and the means were compared using the test of Student Newman-keuls to the probability level p<0.05. The curves were obtained using Microsoft Excel 2013.

**RESULTS**

**Microbial populations during the processing of zoom-koom from fermented and unfermented millet or red sorghum dough**

**Zogona production sites**

Table 1 presents the evolution of microbial populations associated to the processing of zoom-koom produced from fermented and unfermented millet or red sorghum dough from the raw grains to the final products (zoom-koom) ready to drink. The counts of enterobacteria, yeasts, LAB and total mesophilic cells were respectively 2.7×10^3, 1.5×10^4, 1.4×10^3, and 2.3×10^6 CFU/g in the millet grains and 2.9×10^5, 6.3×10^5, 5.9×10^5 and 1.7×10^7 CFU/g in the red sorghum grains. During the soaking steps, a significant increase of enterobacteria, yeasts and LAB counts was observed (p<0.05). However, during the fermentation of the millet dough, a significant decreasing of the enterobacteria and yeasts counts was observed (p<0.05), respectively from 8.7×10^7 to 3.2×10^5 CFU/g and 4.4×10^6 to 8.4×10^3 CFU/g. For the fermentation of the red sorghum dough, the enterobacteria counts were decreased significantly (p<0.05) from 4.7×10^6 to 4.8×10^4 CFU/g, while the yeasts counts followed a non-significant increase (p<0.05) from 5.5×10^4 to 7.6×10^4 CFU/g. The LAB counts were increased (from 2.2×10^8 to 5.6×10^8 CFU/g for millet dough and from 8.9×10^5 to 5.0×10^10 CFU/g for the red sorghum dough) and remain the main flora during the fermentation step of the millet and red sorghum dough (r=0.9). No significant difference (p<0.05) was observed between unfermented zoom-koom without sugar and unfermented zoom-koom with sugar and tamarind juice, except for the enterobacteria counts from unfermented red sorghum zoom-koom.

**Dassasgho production site**

Table 2 presents the evolution of microbial populations associated to the processing of zoom-koom produced from fermented and unfermented millet or red sorghum dough, from the raw grains to the final products (zoom-koom) ready to drink. The counts of enterobacteria, yeasts, LAB and total mesophilic cells were, respectively 8.4×10^4, 1.5×10^4, 8.2×10^4 and 5.1×10^6 CFU/g in the millet grains, and 2.9×10^6, 1.8×10^5, 1.6×10^4 and 5.0×10^5 CFU/g in the red sorghum grains, respectively. During the soaking steps, a significant increase of enterobacteria,
Table 1. Microbial populations during the production of millet or sorghum zoom-koom at Zogona sites.

| Sample          | Products                          | Microorganisms count (cfu/g) | Total mesophilic cells | Enterobacteria | Yeasts | Lactic acid bacteria |
|-----------------|-----------------------------------|------------------------------|------------------------|----------------|--------|---------------------|
|                 |                                   | Millet | Sorghum | Millet | Sorghum | Millet | Sorghum | Millet | Sorghum | Millet | Sorghum | Millet | Sorghum |
| Steps of sampling |                                    |        |         |        |         |        |         |        |         |        |         |        |         |
| Soaked Grains   | Raw grains                         | (2.3 ± 0.1)10³ᵃ | (1.7 ± 1.5)10³ᵇ | (2.7 ± 1.0)10³ᵇ | (2.9 ± 0.6)10³ᵇ | (1.5 ± 1.1)10³ᵃ | (6.3 ± 0.1)10³ᵇ | (1.4 ± 0.1)10³ᵇ | (5.9 ± 5.9)10³ᵇ |
|                 | Start of soaking                   | (8.2 ± 1.8)10³ᵃ | (4.7 ± 3.5)10³ᵇ | (1.9 ± 1.7)10³ᵇ | (1.6 ± 0.9)10³ᵇ | (3.3 ± 0.3)10³ᵇ | (5.4 ± 0.4)10³ᵇ | (2.0 ± 2.0)10³ᵇ | (2.3 ± 1.2)10³ᵇ |
|                 | End of soaking                     | (1.5 ± 1.5)10³ᵃᵇ | (4.3 ± 4.3)10³ᵇ | (2.0 ± 1.4)10³ᵇ | (3.4 ± 3.4)10³ᵇ | (6.7 ± 5.3)10³ᵇ | (2.0 ± 1.8)10³ᵇ | (3.7 ± 0.5)10³ᵇ | (7.5 ± 7.4)10³ᵇ |
| Fermented samples | Start of fermentation              | (4.4 ± 2.1)10³ᵇ | (1.8 ± 1.4)10³ᵇ | (8.7 ± 6.1)10³ᵃ | (4.7 ± 2.6)10³ᵃ | (4.4 ± 3.3)10³ᵇ | (5.5 ± 1.4)10³ᵇ | (2.2 ± 1.2)10³ᵇ | (8.9 ± 7.4)10³ᵇ |
|                 | End of fermentation                | (9.7 ± 0.03)10³ᵇ | (6.0 ± 0.5)10³ᵃ | (3.2 ± 3.1)10³ᵇ | (4.8 ± 2.5)10³ᵇ | (6.4 ± 1.3)10³ᵇ | (7.6 ± 0.7)10³ᵇ | (5.6 ± 0.9)10³ᵇ | (5.0 ± 0.9)10³ᵇ |
| Zoom-koom       | Without sugar                      | (1.2 ± 0.1)10³ᵇ | (5.3 ± 0.9)10³ᵇ | (8.6 ± 6.8)10³ᵇ | (4.1 ± 2.4)10³ᵇ | (1.7 ± 0.4)10³ᵇ | (4.6 ± 0.9)10³ᵇ | (8.4 ± 0.1)10³ᵇ | (2.2 ± 0.4)10³ᵇ |
|                 | With sugar                         | (2.0 ± 0.3)10³ᵇ | (1.6 ± 0.3)10³ᵇ | (8.8 ± 7.4)10³ᵇ | (4.4 ± 2.9)10³ᵇ | (2.2 ± 0.4)10³ᵇ | (4.4 ± 0.7)10³ᵇ | (2.6 ± 0.4)10³ᵇ | (1.5 ± 0.02)10³ᵇ |
| Dough           |                                   | (4.4 ± 2.1)10³ᵇ | (1.8 ± 1.4)10³ᵇ | (8.7 ± 6.1)10³ᵃ | (4.7 ± 2.6)10³ᵃ | (4.4 ± 3.3)10³ᵇ | (5.5 ± 1.4)10³ᵇ | (2.2 ± 1.2)10³ᵇ | (8.9 ± 7.4)10³ᵇ |
| Unfermented samples | Without sugar                      | (1.7 ± 0.1)10³ᵇ | (1.1 ± 0.7)10³ᵃ | (2.8 ± 0.9)10³ᵃ | (2.3 ± 0.04)10³ᵃ | (1.2 ± 0.5)10³ᵇ | (4.6 ± 0.8)10³ᵃ | (1.1 ± 0.1)10³ᵇ | (5.1 ± 2.8)10³ᵃ |
|                 | With sugar and Tamarind            | (4.4 ± 4.3)10³ᵇ | (2.4 ± 0.9)10³ᵃ | (6.5 ± 6.2)10³ᵃ | (2.2 ± 0.2)10³ᵇ | (4.0 ± 3.6)10³ᵇ | (1.5 ± 1.1)10³ᵇ | (2.0 ± 2.0)10³ᵇ | (7.6 ± 4.2)10³ᵇ |

Each column values having a common letter are not significantly different according to the Student Newman Keuls test at the 5% threshold.

Table 2. Microbial population during the production of millet or sorghum zoom-koom at Dassagho production site.

| Sample          | Products and Steps of sampling | Microorganisms count (cfu/g) | Total mesophilic cells | Enterobacteria | Yeasts | Lactic acid bacteria |
|-----------------|--------------------------------|------------------------------|------------------------|----------------|--------|---------------------|
|                 |                                | Millet | Sorghum | Millet | Sorghum | Millet | Sorghum | Millet | Sorghum | Millet | Sorghum | Millet | Sorghum |
| Soaked Grains   | Raw grains                      | (5.1 ± 3.9)10³ᵇ | (5.0 ± 0.5)10³ᵃ | (8.4 ± 7.5)10³ᵃ | (1.2 ± 0.1)10³ᵃ | (1.5 ± 1.1)10³ᵇ | (1.8 ± 0.2)10³ᵇ | (8.2 ± 5.8)10³ᵇ | (1.6 ± 0.1)10³ᵇ |
|                 | Start of soaking                | (4.0 ± 2.1)10³ᵇ | (1.3 ± 0.1)10³ᵃ | (1.7 ± 1.6)10³ᵃ | (4.7 ± 0.2)10³ᵇ | (3.3 ± 0.3)10³ᵇ | (1.3 ± 0.7)10³ᵇ | (1.0 ± 0.9)10³ᵇ | (1.5 ± 0.5)10³ᵇ |
|                 | End of soaking                  | (1.9 ± 0.5)10³ᵃ | (2.2 ± 0.5)10³ᶜ | (5.3 ± 4.6)10³ᵃ | (1.0 ± 0.1)10³ᵃ | (1.6 ± 1.2)10³ᵇ | (1.1 ± 1.0)10³ᵇ | (1.6 ± 0.6)10³ᵇ | (5.0 ± 0.5)10³ᶜ |
| Fermented samples | Start of fermentation            | (7.8 ± 3.2)10³ᵃ | (1.3 ± 0.6)10³ᵇ | (9.1 ± 2.8)10³ᵃ | (2.7 ± 0.1)10³ᶜ | (1.8 ± 0.5)10³ᵇ | (4.4 ± 2.3)10³ᵃ | (7.1 ± 4.9)10³ᵃ | (3.4 ± 0.2)10³ᵇ |
|                 | End of fermentation             | (8.8 ± 2.1)10³ᵃ | (3.2 ± 0.7)10³ᵃ | (1.8 ± 0.7)10³ᵃ | (8.5 ± 0.4)10³ᵇ | (1.8 ± 1.0)10³ᵇ | (2.1 ± 0.9)10³ᵇ | (7.1 ± 1.7)10³ᵇ | (2.1 ± 0.3)10³ᵃ |
| Zoom-koom       | Without sugar                    | (6.0 ± 3.6)10³ᵃ | (5.9 ± 0.7)10³ᶜ | (1.4 ± 1.3)10³ᵃ | (1.2 ± 0.1)10³ᶜ | (1.7 ± 1.5)10³ᵇ | (2.7 ± 0.6)10³ᵃ | (5.0 ± 3.0)10³ᵇ | (3.2 ± 1.3)10³ᵇ |
|                 | With sugar                       | (1.8 ± 0.1)10³ᵃ | (3.5 ± 1.0)10³ᶜ | (9.2 ± 8.7)10³ᵃ | (1.3 ± 0.1)10³ᶜ | (2.5 ± 2.2)10³ᵇ | (1.5 ± 0.3)10³ᵇ | (8.0 ± 6.0)10³ᵇ | (2.6 ± 0.5)10³ᶜ |
| Dough           |                                   | (7.8 ± 3.2)10³ᵃ | (1.3 ± 0.6)10³ᵇ | (9.1 ± 2.8)10³ᵃ | (2.7 ± 0.1)10³ᵇ | (1.8 ± 0.5)10³ᵇ | (4.4 ± 2.3)10³ᵃ | (7.1 ± 4.9)10³ᵃ | (3.4 ± 0.2)10³ᵇ |
| Unfermented samples | Without sugar                    | (3.4 ± 2.4)10³ᵇ | (9.4 ± 2.6)10³ᵇ | (2.4 ± 0.1)10³ᵇ | (1.9 ± 0.5)10³ᵇ | (5.1 ± 1.1)10³ᵇ | (2.1 ± 0.9)10³ᵇ | (2.4 ± 1.9)10³ᵇ | (5.1 ± 0.9)10³ᵇ |
|                 | With sugar and Tamarind          | (4.9 ± 2.7)10³ᵇ | (2.3 ± 0.6)10³ᶜ | (2.2 ± 1.2)10³ᵇ | (2.6 ± 2.2)10³ᶜ | (5.3 ± 1.9)10³ᵇ | (1.1 ± 0.3)10³ᶜ | (2.6 ± 2.1)10³ᵇ | (7.8 ± 0.4)10³ᶜ |

Each column values having a common letter are not significantly different according to the Student Newman Keuls test at the 5% threshold.
yeasts and LAB counts was observed (p<0.05). The results from Table 2 also show a non-significant decrease (p<0.05) of the enterobacteria counts during the fermentation steps of the millet dough (from 9.1×10^6 to 1.8×10^6 CFU/g). A significant increase (p<0.05) of enterobacteria counts during the fermentation of red sorghum dough (from 2.7×10^6 to 8.5×10^6 CFU/g). The yeast counts followed a non-significant decrease (p<0.05) during the fermentation of red sorghum dough (from 4.4×10^5 to 2.1×10^5 CFU/g) and a stability for the fermented dough of millet (1.8×10^5 to 1.8×10^5 CFU/g). The LAB which remain the main flora (r=0.8) faced a significant raise during the fermentation (from 7.1×10^7 to 7.1×10^6 CFU/g and from 3.4×10^7 to 2.1×10^6 CFU/g, respectively for millet and red sorghum dough). A comparison between fermented and unfermented millet and red sorghum zoom-koom, showed that the unfermented zoom-koom of millet and red sorghum contain almost the same counts of enterobacteria and yeasts. But, the fermented millet dough contains less enterobacteria than the fermented dough of red sorghum. A significant difference between the unfermented zoom-koom without sugar and the unfermented zoom-koom with sugar and tamarind juice was observed (p<0.05), except for the enterobacteria counts from unfermented red sorghum zoom-koom and LAB counts from unfermented millet zoom-koom.

### Evaluation of physico-chemical parameters during the processing of zoom-koom from fermented and unfermented millet or red sorghum dough

**Zogona production site**

Table 3 presents the evaluation of pH, titratable acidity and the temperature associated to the processing of zoom-koom produced from fermented and unfermented millet or red sorghum dough. The pH and titratable acidity of the grains were respectively 6.2 and 0.20 g/100 g (as lactic acid) for the millet grain and also respectively 6.4 and 0.06 g/100 g (lactic acid) for the red sorghum grain. During the soaking step of the grains, the pH of the soaking water decreased significantly (p<0.05) from 6.2 to 4.7 and from 6.2 to 5.1, respectively for millet and sorghum grain. Therefore, during the fermentation of the dough, a significant decrease (p<0.05) in pH from 5.7 to 4.1 and from 6.2 to 4.2, respectively for fermented millet dough and fermented red sorghum dough. The titratable acidity calculated as lactic acid content raised significantly (p<0.05) from 0.45 to 0.71 g/100 g and from 0.15 to 0.49 g/100 g, respectively for fermented millet dough and fermented red sorghum dough. The pH of the unfermented zoom-koom had shown a non-significant decrease except for the millet zoom-koom. The titratable acidity has also shown a non-

### Table 3. Physico-chemical parameters of samples during the processing of millet or sorghum zoom-koom at Zogona production site.

| Sample          | Products and steps of sampling | pH      | Titratable acidity (Lactic acid g/100 g) | Temperature (°C) |
|-----------------|--------------------------------|---------|----------------------------------------|-----------------|
|                 |                                | Millet  | Sorghum | Millet  | Sorghum | Millet  | Sorghum | Millet  | Sorghum |
| Raw Grains      |                                | 6.2 ± 0.0a | 6.4 ± 0.3a | 0.20 ± 0.15a | 0.06 ± 0.01d | nd      | nd      |         |         |
| Start of soaking|                                | 6.2 ± 0.1a | 6.2 ± 0.2a | 0.10 ± 0.06a | 0.08 ± 0.01d | nd      | nd      |         |         |
| End of soaking  |                                | 4.7 ± 0.4a | 5.1 ± 0.1b | 0.22 ± 0.01a | 0.13 ± 0.02d | nd      | nd      |         |         |
| Start of fermentation |                            | 5.7 ± 0.0a | 6.2 ± 0.1a | 0.45 ± 0.15a | 0.15 ± 0.01cd | 26.5 ± 1.5a | 26.0 ± 0.0d |
| End of fermentation |                            | 4.1 ± 0.0a | 4.2 ± 0.0c | 0.71 ± 0.28a | 0.49 ± 0.05a | 29.5 ± 1.5b | 26.5 ± 0.5c |
| Zoom-koom       | Without sugar                  | 3.9 ± 0.1c | 4.2 ± 0.0c | 0.25 ± 0.15a | 0.25 ± 0.03bc | nd      | nd      |         |         |
| With sugar      |                                | 3.8 ± 0.0a | 4.2 ± 0.0c | 0.39 ± 0.25a | 0.33 ± 0.07bc | nd      | nd      |         |         |
| Zoom-koom       | Dough                          | 5.7 ± 0.0a | 6.2 ± 0.1a | 0.45 ± 0.15a | 0.15 ± 0.01a  | nd      | nd      |         |         |
| Without sugar   |                                | 5.5 ± 0.3a | 6.3 ± 0.0a | 0.19 ± 0.12a | 0.12 ± 0.04a  | nd      | nd      |         |         |
| With sugar and Tamarind |              | 3.9 ± 0.1b | 4.5 ± 1.0a | 0.24 ± 0.11d | 0.26 ± 0.09a  | nd      | nd      |         |         |

*Each column values having a common letter are not significantly different according to the Student Newman Keuls test at the 5% threshold. nd: Not determined.*
significant slight increase after tamarind juice adding. The pH of zoom-koom based on fermented millet or red sorghum dough was almost similar to those of unfermented zoom-koom mixed with tamarind juice. During the fermentation, the temperature of the dough showed a significant variation (p<0.05) for fermented millet dough (from 26.5 to 29.5°C) and a slight non-significant variation (p>0.05) for the fermented red sorghum dough (from 26 to 26.5°C). It is noticed that fermentation using red sorghum dough is slower than fermentation using millet dough. This led to less important reduction of pH than for millet dough.

**Dassasgho production site**

Table 4 presents the values of pH, titratable acidity and the temperature associated to the processing of zoom-koom produced from fermented and unfermented millet or red sorghum dough. The pH and the titratable acidity of the grains were respectively 6.2 and 0.10 lactic acid g/100 g for the millet grain and also respectively 6.2 and 0.07 lactic acid g/100 g for the red sorghum grain. During the soaking steps of the grains, the pH was decreased non-significantly (p>0.05) from 6.3 to 5.4 and significantly from 6.1 to 4.5, respectively for millet and sorghum grain. During the fermentation, the pH of the fermented millet dough showed a significant decrease (p<0.05) from 5.7 to 4.2, but the pH of red sorghum dough showed a significant decrease (from 5.8 to 4.6), but less important than that of millet dough. The titratable acidity calculated as lactic acid content increased significantly (p<0.05) from 0.37 to 0.90 g/100 g and from 0.37 to 0.45 g/100 g, respectively for fermented millet and red sorghum dough. The pH of the unfermented zoom-koom had shown a non-significant decrease excepted for the red sorghum zoom-koom. The lactic acidity had also shown a non-significant increase after tamarind juice adding. The pH of zoom-koom based on fermented millet or red sorghum dough was slightly similar to those of unfermented zoom-koom mixed with tamarind juice. The fermentation temperature showed a non-significant variation (p>0.05) for fermented millet dough (from 21.0 to 21.5°C) and for red sorghum dough (from 21.5 to 22°C). It is noticed that fermentation using red sorghum dough is slower than fermentation using millet dough. This lead to less important reduction of pH than for millet dough.

Main presumptive LAB isolates collected from the zoom-koom production at the two productions sites

A total of 350 presumptive isolates of LAB (Gram +, catalase -, oxidase -, rods or cocci) have been collected from the samples taking at zoom-koom production process (Tables 5 and 6). The production made in the district of Zogona allowed

| Sample          | Products and steps of sampling | pH       | Titratable acidity (Lactic acid g/100g) | Temperature (°C) |
|-----------------|--------------------------------|----------|----------------------------------------|------------------|
|                 |                                | Millet   | Sorghum                               | Millet           |
|                 |                                |          |                                        | Sorghum          |
|                 |                                |          |                                        | Millet           |
|                 |                                |          |                                        | Sorghum          |
| Fermented samples | Grain                          | 6.2 ± 0.0<sup>a</sup> | 6.2 ± 0.0<sup>a</sup> | 0.10 ± 0.03<sup>b</sup> | 0.07 ± 0.01<sup>c</sup> | nd | nd |
|                 | Start of soaking                | 6.3 ± 0.0<sup>a</sup> | 6.1 ± 0.0<sup>b</sup> | 0.10 ± 0.03<sup>b</sup> | 0.04 ± 0.01<sup>c</sup> | nd | nd |
|                 | End of soaking                  | 5.4 ± 0.4<sup>a</sup> | 4.5 ± 0.3<sup>b</sup> | 0.15 ± 0.05<sup>b</sup> | 0.10 ± 0.01<sup>c</sup> | nd | nd |
|                 | Start of fermentation           | 5.7 ± 0.5<sup>a</sup> | 5.8 ± 0.1<sup>a</sup> | 0.37 ± 0.06<sup>b</sup> | 0.13 ± 0.01<sup>c</sup> | 21.0 ± 1.0<sup>a</sup> | 21.5 ± 0.5<sup>a</sup> |
|                 | End of fermentation             | 4.2 ± 0.0<sup>b</sup> | 4.6 ± 0.1<sup>b</sup> | 0.90 ± 0.14<sup>a</sup> | 0.45 ± 0.05<sup>a</sup> | 21.5 ± 0.5<sup>a</sup> | 22.0 ± 0.0<sup>a</sup> |
| Zoom-koom       | Without sugar                   | 4.2 ± 0.1<sup>b</sup> | 4.9 ± 0.1<sup>b</sup> | 0.36 ± 0.04<sup>b</sup> | 0.25 ± 0.05<sup>b</sup> | nd | nd |
|                 | With sugar                      | 4.1 ± 0.1<sup>b</sup> | 4.9 ± 0.0<sup>b</sup> | 0.34 ± 0.08<sup>b</sup> | 0.15 ± 0.05<sup>c</sup> | nd | nd |
| Unfermented samples | Dough                          | 5.7 ± 0.5<sup>a</sup> | 5.8 ± 0.1<sup>a</sup> | 0.37 ± 0.06<sup>b</sup> | 0.13 ± 0.01<sup>a</sup> | nd | nd |
| Zoom-koom       | Without sugar                   | 5.2 ± 0.8<sup>a</sup> | 6.0 ± 0.1<sup>a</sup> | 0.17 ± 0.04<sup>a</sup> | 0.07 ± 0.01<sup>a</sup> | nd | nd |
|                 | With sugar and Tamarind         | 3.5 ± 0.1<sup>a</sup> | 3.8 ± 0.0<sup>b</sup> | 0.40 ± 0.04<sup>a</sup> | 0.15 ± 0.05<sup>a</sup> | nd | nd |

Each column values having a common letter are not significantly different according to the Student Newman Keuls test at the 5% threshold. nd: Not determined.
Table 5. Distribution of LAB isolates from the raw grains to the final product throughout the processing of millet and red sorghum zoom-koom at Zogona production site.

| Sample                                | Cocci in pair and tetrad | Cocci in pair and short chains | Long rods in pair and short chains | Short rods in pair and short chains | Cocci in pair and tetrad | Cocci in pair and short chains | Long rods in pair and short chains | Short rods in pair and short chains | Total of isolates collected by Samples |
|----------------------------------------|--------------------------|--------------------------------|----------------------------------|-----------------------------------|--------------------------|--------------------------------|----------------------------------|-----------------------------------|---------------------------------------|
| Raw Grains                             | -                        | -                              | -                                | -                                 | -                        | -                              | -                                | -                                 | 8                                     |
| Soaked Grains, Start of soaking         | -                        | 6                              | -                                | 2                                 | 6                        | -                              | -                                | -                                 | 8                                     |
| Soaked Grains, End of soaking           | 1                        | 3                              | 1                                | 4                                 | 1                        | -                              | -                                | -                                 | 10                                    |
| Soaking Water, start of soaking         | -                        | 7                              | -                                | -                                 | -                        | -                              | -                                | -                                 | 7                                     |
| Soaking Water, end of soaking           | -                        | 2                              | 1                                | 6                                 | -                        | -                              | -                                | -                                 | 9                                     |
| Dough, start of fermentation            | -                        | -                              | 1                                | 11                                | -                        | -                              | -                                | -                                 | 9                                     |
| Dough, end of fermentation              | -                        | -                              | 1                                | 12                                | -                        | -                              | -                                | 1                                 | 10                                    |
| Fermented zoom-koom without sugar      | -                        | -                              | 1                                | 11                                | -                        | 1                              | 2                                | 12                                | 27                                    |
| Fermented zoom-koom with sugar         | -                        | -                              | 1                                | 21                                | -                        | -                              | -                                | -                                 | 18                                    |
| Unfermented zoom-koom without sugar    | -                        | -                              | 2                                | 11                                | 2                        | 1                              | -                                | -                                 | 11                                    |
| Unfermented zoom-koom with sugar and tamarind | 1                  | 3                              | 2                                | 6                                 | 2                        | 2                              | -                                | -                                 | 16                                    |
| Total                                  | 3                        | 23                             | 7                                | 80                                | 11                       | 4                              | 3                                | 76                                | 207                                   |

*: Not found.

To collect 207 presumptive LAB isolates. From the district of Dassasgho, a total of 143 presumptive LAB isolates were collected. The isolates were cocci in pair and tetrad, cocci in pair and short chains, long rods in pair and short chains, short rods in pair and short chains. The results shown that presumptive LAB associated to the production of the millet and red sorghum zoom-koom were dominated by short rods: 70.79 and 80.85%, respectively for millet zoom-koom and red sorghum zoom-koom in Zogona production site (Table 5); 91.56 and 86.66%, respectively for millet zoom-koom and red sorghum zoom-koom, in the district of Dassasgho (Table 5). Further works will be focused on the characterization and the identification of the isolates and will give more informations on the microbial species involved.

**DISCUSSION**

The microorganisms (yeasts, moulds, enterobacteria, and LAB) present in the raw grains of millet and red sorghum represent the endogenous flora of these grains. During the soaking steps, the decrease in pH and the increase in titratable acidity can be attributed to the activities of LAB, which population increased during this step. It means that a natural lactic fermentation occurred during the soaking step of the millet and sorghum grains. This has been reported by previous investigations (Sawadogo-Lingani et al., 2010).

The increase of enterobacteria and yeasts counts during the soaking could be due to the availability of nutrients and the increase in moisture content. Moreover, some studies conducted on the sorghum have shown that the soaking is a very critical step in the malting of sorghum (Tawaba et al., 2013). In fact, the growth of the microorganisms present in the grains during the soaking step, may be due to the favorable moisture content (35-40% or even more) (Ogbonna et al., 2004) and therefore the activity of the water, thus creating conditions favorable to the activation of the spores and the development of the bacteria, yeasts and molds (Tawaba et al., 2013).

The decrease in enterobacteria and yeasts counts during the fermentation of millet and red
Table 6. Distribution of LAB isolates from the raw grains to the final product throughout the processing of millet and red sorghum zoom-koom at Dassasgho production site.

| Sample                                    | Dassasgho | Sorghum | Total of isolates collected by samples |
|-------------------------------------------|-----------|---------|---------------------------------------|
|                                           | Millet    | Sorghum |                                        |
|                                           | Cocci in pair and tetrad | Cocci in pair and short chains | Long rods in pair and short chains | Short rods in pair and short chains | Cocci in pair and tetrad | Cocci in pair and short chains | Long rods in pair and short chains | Short rods in pair and short chains |
| Raw grains                                | -         | -       | -                                     | -                                   | -         | -       | -                                     | -                                   |
| Soaked grains, start of soaking           | 2         | -       | -                                     | 2                                   | 1         | -       | -                                     | 2                                   |
| Soaked grains, end of soaking             | -         | 2       | 3                                     | -                                   | 1         | -       | -                                     | 1                                   |
| Soaking Water, start of soaking           | -         | -       | 5                                     | -                                   | -         | -       | -                                     | 3                                   |
| Soaking Water, end of soaking             | 1         | -       | 12                                    | -                                   | -         | -       | -                                     | 13                                  |
| Dough, start of fermentation              | -         | -       | 9                                     | 1                                   | -         | 1       | 6                                     | 17                                  |
| Dough, end of fermentation                | 1         | 1       | 12                                    | -                                   | -         | -       | 10                                    | 24                                  |
| Fermented zoom-koom without sugar        | -         | -       | 4                                     | -                                   | -         | -       | 8                                     | 12                                  |
| Fermented zoom-koom with sugar           | -         | -       | 8                                     | -                                   | -         | 1       | 9                                     | 18                                  |
| Unfermented zoom-koom without sugar      | -         | -       | 11                                    | -                                   | -         | 1       | 8                                     | 20                                  |
| Unfermented zoom-koom with sugar and tamarind | -       | -       | 12                                    | -                                   | 2         | 10      | 24                                    |
| Total                                     | 4         | 1       | 2                                     | 76                                  | 2         | 1       | 5                                     | 52                                  | 143                                |

Sorghum dough is probably due to the effect of the acidification of the dough (decrease in pH and the increase in titratable acidity) resulting to the concomitant growth of LAB during this step. These results corroborate with those of Vieira-Dalodé et al. (2008) in the production of gowè, a sour beverage from Benin, who found a significant increase of LAB count after 4 h of fermentation and during the fermentation step as well as a significantly lower count of yeasts. Our results are also in line with those of Soma (2014) in the production of zoom-koom, Sawadogo-Lingani et al. (2007) in the production of dolo and pito, Lei and Jakobsen (2004) in the production of koko and Muyanja et al. (2002) in the production of bushera, who found a LAB counts of $10^8$ and $10^9$ cfu/g during the fermentation process. Sawadogo-Lingani et al. (2010) in the traditional malting of sorghum had found a LAB counts which increased from $10^5$ to $10^{10}$ cfu/g during the steeping of the sorghum grains.

In fact, LAB are well known to be producers of natural antimicrobial substances like organic acids (lactic, acetic, formic, phenyllactic caproic), carbon dioxide, hydrogen peroxide, ethanol and bacteriocins (Messens and De Vuyst, 2002). The production of organic acid during the fermentation process induce an important decrease in pH, which in association with the formation of antibacterial substances determine the microbial stability as well as the level of the growth of pathogenic bacteria and other undesirable microorganisms. Previous works showed that the organic acids produced during the fermentation of pito in Ghana, tchapalo in Ivory Coast and ben-saalga in Burkina Faso, helped to obtain a better microbial stability of the products (Tou et al., 2006; Dje et al., 2008), which can be also applied for zoom-koom. LAB are able to synthesize some active bacteriocins not only against other lactic bacteria, but also against other gram positive bacteria and moulds as well as against gram negative bacteria, among which enterobacteria and pathogenic germs (Raimbiault, 1995) are important. It can be noted that the dilution (addition of water) reduced the microbial counts. The growth of yeasts during the fermentation of red sorghum dough may be partially attributed to the presence of lactic acid. Our results are similar to those obtained by Mohammed et al. (1991), who found a high number of yeasts during the
fermentation of sorghum. These results can explain the increase in yeasts counts during the fermentation of sorghum dough at Zogona’s site and its decrease during the fermentation at Dassagho’s site. The difference in the number of LAB between millet and sorghum dough during the fermentation in Zogona production site could be explained by the fact that at the start of sorghum fermentation the number of LAB was low, resulting in less competition. When this number rises it could have more competition and fewer sugars available.

The stationary state of enterobacteria counts and the increase in yeasts counts during the fermentation process at Dassagho site may be due in part to the low acidification of the dough and in other hand to the low temperature of fermentation (21 to 22°C) because of the climatic factors in that periods (July and August with a room temperature around 33°C (period of the raining season in Burkina Faso). If the initial acidification is not fast enough, some pathogenic or undesirable germs can partially develop, maintain themselves and deviate or evolve later the product, during the conservation period. For this reason, it is recommended to assure that the initial phase of acidification is sufficiently fast for the good microbiological quality of product (Raimbiault, 1995). Some studies showed that, for an efficacy stable action, the acidification may be fast, in less than 24 h, and the pH may drop below 4.0 (Raimbiault, 1995). According to Muyanja et al. (2002), a pH of 3.5 to 4.0 has been reported to inhibit Enterobacteriaceae and other Gram-negative bacteria. The results found in the production of Dassasgho corroborate with those of Brisabois et al. (1997) on milk pathogens which found that a small variation in pH can reduce (pH 4.55) or promote (pH 4.95) the development of pathogens (Brisabois et al., 1997). Indeed, studies on milk pathogens carried out in France and in Europe have concluded that most pathogenic bacteria develop at pH intervals of between 4.5 and 9.6 (Brisabois et al., 1997). These results corroborate with those found during the fermentation realized in Dassasgho production site, where the pH for the fermentation of red sorghum dough was decreased from 5.8 to 4.6 resulting in a small reduction in enterobacteria counts.

The slowness of the fermentation of red sorghum dough compared to the millet dough could be due to the structural composition of the two grains. The red sorghum is rich in tannins. This compound impact the enzymatic digestibility, thereby, the availability of sugars which are the basic substrate for lactic fermentation. Studies also showed that those phenolic compounds in one hand are capable of protecting the grains against fungus attack, insects and birds, which is an obvious agronomic advantage, reducing the enzymatic digestion as well as the proteins, starch and others polysaccharides (Tawaba et al., 2013). This slowness of the fermentation causes a less important drop of the pH of zoom-koom and also a less important production of lactic acid compared to the fermented zoom-koom realized with millet dough. The enterobacteria counts are lower in the sorghum zoom-koom for some case like Zogona’s productions. This result could be explained by the low acidity. The most of the isolates involved in the fermentation process of sorghum and millet grains for the production of zoom-koom, are Gram positive, catalase negative and oxydase negative. Rods in pair and short chains could belong to the genus of Lactobacillus. These partial results corroborate with previous studies (Sawadogo-Lingani et al., 2007; Lei and Jakobsen et al., 2004) where Lactobacillus species had been identified as dominant LAB involved in the fermentation of sorghum (dolo, pito) or millet based products (koko).

Conclusion

The present study highlighted the importance of fermentation in food processing by improving the microbiological quality of final products. During the processes of millet and red sorghum zoom-koom, the fermentation of the dough before the filtration steps was able to reduce the number of enterobacteriea and yeasts. Fermentations taking place under low temperatures need more fermentation time for a better acidification of the dough. Fermented zoom-koom is better than unfermented zoom-koom, in terms of microbiological quality for the case of millet. In the case of sorghum zoom-koom, the unfermented zoom-koom is better than fermented zoom-koom. Fermented millet zoom-koom is better than fermented sorghum zoom-koom, but the unfermented sorghum zoom-koom is better than unfermented millet zoom-koom in terms of microbiological quality. LAB isolates of the genus of Lactobacillus seem to be the dominant bacteria involved in the fermentation of zoom-koom. The identification of the isolates and the determination of their technological properties are essential for the optimization of fermentation processes and the improvement of the quality of the final products by the use of selected LAB starter cultures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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