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Characterization of *Massa* (a fermented millet pancake type food) production process and isolation of exopolysaccharides producing lactic acid bacteria

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Abstract

Massa is a traditional ready to eat pancake-type food made from fermented millet dough. In this study, processing of *massa* produced from whole millet grains and the microorganisms associated were assessed. Flow diagrams *of massa* production were determined in two production sites in the city of Ouagadougou in Burkina Faso. Samples, from raw material to end-products, were collected and physicochemical parameters and microbiological characteristics were evaluated using standards methods. Lactic acid bacteria (LAB) were isolated, characterized and screened for exopolysaccharides production. The average mean values of pH and titratable acidity respectively were 6.41±0.01 and 0.35±0.04 (lactic acid g/100 g) for the raw material; and evolved during fermentation of the dough from 4.93±1.36 to 4.02± 0.21 and from 1.17± 0.18 to 1.38± 0.02 (lactic acid g/100 g) and for *massa* were 4.49±0.52 and 0.46±0.11 (lactic acid g/100 g). Aerobic mesophilic bacteria, enterobacteria, LAB, yeasts and molds counts, evolved during fermentation respectively from 7.35±1.64 to 9.13±0.47 log CFU.g^{-1}; from 4.77±0.80 to 1.64±0.34 log CFU.g^{-1}; from 6.29±1.03 to 9.05±0.29 log CFU.g^{-1} and from 4.82±1.61 to 4.53±0.24 log CFU.g^{-1}*Massa* was characterized by a water content ranging from 39.39 to 42.09 % and a low microbiological load ranging from 1.60 to 2.36 log CFU.g^{-1} for aerobic mesophilic bacteria counts and less than 1.00 log CFU.g^{-1} for enterobacteria, lactic acid bacteria, yeasts and molds. Microbiological analysis showed that *massa* samples had an acceptable hygienic quality. Among 186 presumptive LAB observed, 5 strains with high exopolysaccharides (EPS) production were found.

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Introduction

In Burkina Faso, cereals are staple food and their annual consumption is estimated to account for 62% of food consumed by households (DGPER, 2009). The processing of cereals provides several types of products. For instance, sorghum (*Sorghum bicolor*), maize (*Zea maize*) and millet (*Pennisetum glaucum*) are processed into alcoholic and non-alcoholic beverages (*dolo*, *zoom koom*) and various foods such as cooked dough or "*tô*", porridge or "*ben-saalga*", couscous (*bassi*). *Massa* or *womi* is a traditional pancake type food obtained from frying a fermented millet (*Pennisetum glaucum*) dough. *Massa* is produced and marketed locally by women vendors along roads (Konkobo *et al.*, 2002). This food, sweetened, salted or spiced, is commonly found in Burkina Faso, Ghana, Mali, Niger and Nigeria is a ready -to -eat product that can be consumed solely or as an accompaniment to porridges or soups (Nkama, 1998). Consumed within a day and not usually stored *massa* is popular in both urban and rural areas. in Burkina Faso and is mainly consumed.

The process of transforming millet into *massa* involves lactic and/or alcoholic fermentation steps as for most cereal-based fermented products (Sawadogo-Lingani *et al.*, 2007).These fermentations are generally attributed to lactic acid bacteria (LAB) and yeasts, which are the predominant species found in traditional African cereal-based products (Sawadogo-Lingani, 2010). There has been a growing interest in the study of the microbiota associated with fermented traditional foods. Following the various studies, LAB have been isolated and characterized for their technological properties and their use as starter cultures (Sawadogo-Lingani *et al.*, 2008; Agarry *et al.*, 2010) for better control fermentation and standardization of manufacturing processes (Holzapfel, 2002). Several studies on fermented foods have been carried out to assess LAB based on their effect on the nutritional and technological properties to improve food quality(Songré-Ouattara *et al.*, 2010; Sümengen *et al.*, 2012; Florou-paneri *et al.*, 2013).

Due to the increase of coeliac disease involving wheat proteins and chronic diseases such as cancers, and diabetes , dietary fiber carbohydrates have gained attention for their health benefits potentials. However, dehulling is commonly done for most of the grains, thus resulting in a product missing the fraction of nutrients contained in the bran. The main reason of such operation pertains to consumers preferences for refined flour products for sensory attributes such as the colour and the mouthfeel (Awika, 2011). In cereals, the bran is the part rich in in vitamin B group and dietary fiber. Due to their influence on consumer preference and impact on product properties , dietary fiber are considered as sensory attributes and technological properties change factors of food products (Awika, 2011; Piironen, 2011).

Additives, hydrocolloids and enzymes are often added to make whole grain product acceptable for consumers. Such technological aids are usually artificial and contrast with consumers demand for more natural products. There have been a few studies on the use of eps in whole grains. LAB have shown potential through mechanism such as exopolysaccharides production that can act as hydrocolloids and improve the sensory quality of the product (Katina *et al.*, 2009). The potential of LAB can be a better alternative because during their growth LAB lower the pH and that activates indigenous enzymes that can degrade fiber. Better results are obtained when the selected LAB is utilized in a matrice similar to its origin (Coda *et al.*, 2010). Whole grain millet *massa* production can be optimized by selecting suitable stater culture from *massa* production.

Massa being a part of the daily diet of local population in Burkina Faso it is necessary to evaluate this ready to eat fermented food in order to assess the microbiota involved in the processing. To our knowledge, very few studies have been done on *massa* technology as well as on the microorganisms associated with the fermentation of millet dough in the process. In the plan to enhance the utilization of whole grain millet in *massa* technology, this study

was initiated to characterize microorganisms during the production of whole grain millet based *massa* and to select EPS producing LAB for futher applications.

Material and Methods

Study framework and sampling

The work consisted of *massa* production follow ups at two distinct sites located in Zogona and Bilbalogho in the city of Ouagadougou in order to establish flow-diagrams and identify sampling points for laboratory analysis. Samples were collected from *massa* production trials from raw material to finished products for laboratory analyses.

A sample of 100 kg millet grains was purchased at the local market of Zogona and used as raw material by the two producers. For each production, 10 kg of grains were used.

After three follow up of massa processing at the two production sites (Zogona and Bilbalogho), the diagrams of process were established. At each production sites, massa process consisted of four main steps: treatment of the raw material, milling, fermentation of the dough and frying the fermented dough. The main unitary operations are represented in Figs 1 and 2 for Zogona and Bilbalogho *massa* production sites respectively.

All sampling material and utensils was sterilized prior sampling. Each sample consisted of 200 mL or 200g of products for liquid and solid, respectively. Sampling was done in duplicate and transported to the laboratory in a cooler containing ice packs and stored in the refrigerator at 4°C ± 1°C prior to analysis within a maximum of 24 hours.

Physico-chemical analysis

The water content was determined according to NFV03-707 (2000) and results were expressed by conversion into dry matter. Ash contents were determined by differential weighing of 5 g sample after calcination at 550°C for 4 hours according to ISO 2171 (2007). The pH values were measured using a pHmeter (Model HI 8520; Hanna Instrument, Singapore).

For titratable acidity determination, 5 g or 5 mL of sample suspended in 30 mL of ethanol 90% (v/v) were centrifuged for 5 min at 3500 g. From 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 were titrated with 0.1 N NaOH using 1% phenolphthalein 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).

Microbiological analyses

Microorganisms enumeration

From the collected samples, aerobic mesophilic bacteria (AMB), enterobacteria, lactic acid bacteria and yeasts and molds were enumerated using standard methods. For all the analyses, 10 g of the samples were homogenized in a stomacher with 90 ml sterile diluent (0.1% peptone, 0.8% NaCl, pH 7.0 ± 0.2). Tenfold serial dilution was prepared and spread-plated for microorganisms count. Samples were diluted prior to spreading.

For the solid raw material, the product was soaked for 30 min in the diluent at the laboratory temperature (22-25°C), for 30 min before homogenization in the stomacher for 2 min at normal speed.

Aerobic mesophilic bacteria (AMB) were counted by cultivation on pour plates of Plate Count Agar (Liofilchem, Italy) incubated at 30°C for 72 h (ISO 4833, 2003). Yeasts and molds were counted by cultivation on Yeast extract Glucose Chloramphenicol (YGC) agar (HiMedia, India), pH 6.6 ± 0.2, incubated at 30°C for 3 to 5 days according to ISO 7954 (1988). LAB were counted by cultivation on modified Man, Rogosa and Sharpe (MRS-IM agar +4% maltose) (Liofilchem, Italy), incubated anaerobically in an anaerobic jar with anaerocult A at 37°C, for 72 to 96 h according to ISO 15214 (1998) standard. Enterobacteria were enumerated on Violet

Red Bile Glucose (VRBG) agar (Liofilchem, Italy), incubated at 37°C for 24 h according to ISO 7402 (1993).

The results were converted to = log CFU.g^{-1} or mL^{-1} of sample

Isolation, purification and characteristics of LAB: Isolates of lactic acid bacteria were collected from each sample and characterized. For each sample, the highest dilution plates on mMRS growth media were used for the isolation.

Isolation, purification and phenotypical characterization of LAB isolates

For each sample, about 10 colonies were isolated from a representative portion of each plate and purified successively by streaking onto mMRS agar until getting pure colonies. Pure cultures were characterized by colony morphology (size, color, shape, texture, margin and surface type), cell morphology by phase contrast microscope (shape, size, motility, mode of association).

Pure isolates of LAB were confirmed by Gram reaction (Gregersen, 1978) and catalase reaction (3% H_2O_2) prior tests for gas production in glucose-MRS broth with Durham bell. The growth of the selected LAB isolates was tested in MRS broth at pH 2; 4 and 9.6; Vosges Proskauer/Red methyl, amylase and esculin degradation tests were also performed.

Presumptive identification of the LAB isolates at genus level

The presumptive genera of LAB isolates were based on their main characteristics using the information provided in Table 1.

EPS production screening

Presumptive LAB (gram-positive, catalase-negative, oxidase-negative) previously collected, and stored at -80°C in MRS broth (Oxoid, Basingstoke, UK) supplemented with 30% glycerol (Merck) were screened for EPS production. Preliminary screening was carried on LTV agar (0.5 g/L tryptone, 10 g/L meat extract, 6.5 g/L NaCl, 8 g/L potassium nitrate, 8 g/L sucrose, 0.1% (v/v) Tween 80, 17 g/L agar, pH 7.1 ± 0.2) and isolates determined as slimy producers, were selected using the inoculated loop method (Sawadogo-Lingani *et al.*, 2008). In a second step, EPS-positive isolates were inoculated on MRS agar containing 2% (w/v) sucrose where strong slimy producers were selected. In a final step, pearl millet sourdoughs were prepared by mixing 40 parts millet flour with 60 parts distilled water and then fermented with the selected strains according to the method described by Wang *et al.*(2019). Technological parameters such as pH and viscosity (Wang *et al.*, 2019) were evaluated in order to select the best dextran-producing isolates .

EPS producing isolates identification

The genomic DNA of the candidate strain (five isolates) was extracted using a DNeasy blood and tissue kit (Qiagen, France) according to the manufacturer´s instructions. The primer pairs (IDT Technologies, Belgium), LacbF/LacbR, were used for amplifying the 16S rRNA gene fragment (De Angelis *et al.*, 2006). The amplicons were purified with the NucleoSpin® Gel and PCR Clean-up kit and analyzed by agarose gel electrophoresis (1.5% w/v). Taxonomic strain identification was conducted by comparing the 16S rDNA sequence of the isolate with those reported in the NCBI Reference Sequence database (Wang *et al.*, 2019).

Utilization of EPS on massa process

To assess the effect of EPS production on massa processing, a sourdough obtained from the following composition was sent for frying. The millet sourdough was prepared by mixing 50 parts millet flour with 50 parts distilled water, sucrose was added to the mix at 10% The starter culture strain was refreshed for 24h at a temperature of 30°C and inoculated for the preparation of the millet sourdough. The sourdough was incubated at 25°C for 24h.

Statistical analysis

For data from each replication, the mean and standard deviation were calculated and data of microbial counts were converted into log. Statistical significance was performed with one-way univariate

analysis of variance (ANOVA) using XLSTAT 7.5.2, with Turkey's test at the significance $p < 0.05$.

Results and discussion

Massa processing Description of the main stages of massa production

Treatment of the raw material: At Zogona production site, millet grains (10 kg) were cleaned by winnowing, sorted and soaked for 14 ± 2 h; the soaked millet grains were then washed, drained and milled into flour at the local mill. At Bilbalogho, the same quantity of millet grains was also cleaned by winnowing, sorted and washed and then the grains were drained for 5 min, spread out on a mat for a slight sun-drying for 2 h and milled. The millet grains in this study were not dehulled compared to different studies on massa (Nkama and Malleshi, 1998; Ayo *et al.*, 2008; Ayo *et al.*, 2012).

Table 1. Characteristics of main LAB genera isolated from food products.

Family Name	Genus name	Form	Fermentation Type: CO_2 production from glucose	Characteristics							
				Arginine hydrolysis	Growth at 10°C	Growth at 45°C	Growth atNaCl 6,5 %	Growth at N a C l 18 %	Growth atpH 4.4	Growth at pH 9.6	Isomeric lactic acid produced
Aerococcaceae	*Aerococcus*	cocciin tetrads	homofermentative	nd	+	-	+	-	-	+	L
Carnobacteriaceae	*Carnobacterium*	bacilli	heterofermentative	+	+	-	nd	-	nd	-	L
Enterococcaceae	*Enterococcus*	cocci	homofermentative	±	+	+	+	-	+	-	L
	Tetragenococcus	cocci in tetrads	homofermentative	nd	+	-	+	+	±	+	L
	Vagococcus	cocci	homofermentative	±	+	-	-	-	nd	-	L
	Pediococcus	cocciin tetrads	homofermentative	±	±	±	±	-	+	-	L, DL
Lactobacillaceae	*Lactobacillus*	bacilli,coccobacilli	Homoouheterofermentative	±	±	±	±	-	nd		D,L,DL
Leuconostocaecae	*Leuconostoc*	cocci,coccobacilli	heterofermentative	-	+	-	±	-	±	-	D
	Oenococcus	cocci,coccobacilli	heterofermentative	-	+	-	±	-	±	-	D
	Weissella	cocci,bacil	heterofermentative	nd	+	-	±	-	±	-	D,DL
Streptococcaceae	*Lactococcus*	cocci	homofermentative	±	+	-	-	-	±	-	L
	Streptococcus	cocci,,coccobacilli	homofermentative	±	-	±	-	-	-	-	L

Source(Matamoros, 2008; Sawadogo-Lingani, 2010; Liu *et al.*, 2014)

+: positive in 90 %of cases at least; - : negative in 90 %of cases at least; ±: positive/negative depending on species and strains;nd:not determined.

Milling: during the milling process at Zogona, water was added to the grains to produce a dough; but at Bilbalogho, the grains were processed into slightly moist flour. Fermentation of the dough: At Zogona, the dough obtained after milling was kneaded for 5 to 10 min with addition of water and *ben-kida* porridge which had been prepared the day before.

The resulting dough was kneaded to reach the desired consistency, then let to ferment for 5 ± 1h. At Bilbalogho, the flour was kept at room temperature for 5 h and then sieved. A portion of the sieved flour were used to prepare the *ben-saalga* porridge which was obtained by heat gelatinization of a mixture of flour and warm water poured into an equal volume of boiling water. The remaining flour was mixed with added water and *ben-saalga* porridge; the resulting mixture was kneaded/mixed for 5 to 10 min to reach the desired consistency and then let to ferment for 8 to 9 h. The fermentation time at both Zogona and Bilbalogho sites ranges in the (6-12h) applied in Nkama and Malleshi (1998) study.

Table 2. Physicochemical characteristics of samples from Zogona site.

Sample	Ash (% D	Dry matter (%)	Moisture (%)	pH	Titratable acidity (Lactic acid g/100 g DM)
Raw material (Whole millet grains)	1.61± 0.07^{a}	91.63 ± 0.89^{a}	8.37 ± 0.90^{g}	6.42 ± 0.00^{a}	0.32 ± 0.02^{e,f}
Soaked millet (Start)	1.47±0.05^{d}	68.28 ± 0.05^{c}	31.72 ± 0.05^{e}	6.62 ± 0.03^{a}	0.38 ± 0.01^{f,g}
Soaked millet (End)	1.32±0.02^{f}	48.55 ± 0.06^{e}	51.45 ± 0.06^{c}	4.30 ± 0.03^{a,b}	0.74 ± 0.01^{d}
Washed soaked Millet	1.32±0.02^{e}	59.6 ± 0.09^{d}	40.40 ± 0.09^{d}	4.51 ± 0.01^{a,b}	0.65 ± 0.00^{c,d}
Dough from milling	1.20±0.15^{f,g,h}	42.91 ± 0.23^{f,g}	57.09 ± 0.23^{a,b}	4.20 ± 0.01^{b}	0.96 ± 0.02^{c}
Dough at the beginning of fermentation	1.19±0.01^{g,h}	37.88 ± 0.01^{g}	62.12 ± 0.01^{a}	4.00 ± 0.04^{b}	1.04 ± 0.01^{c}
Dough at the end of fermentation	1.23±0.01^{g,h}	37.93 ± 0.07^{g}	62.07 ± 0.07^{a}	3.87 ± 0.01^{b}	1.36 ± 0.01^{b}
Massa (fried fermented dough, finished product)	0.76 ±0.00^{h}	57.91 ± 0.06^{d}	42.09 ± 0.06^{d}	4.12 ± 0.01^{b}	0.54 ± 0.01^{e}

DM: Dry matter; different letters in the same row indicate statistical significance ($p<0.05$).

Frying the fermented dough: At both production sites of Zogona and Bilbalogho, the fermented dough was diluted with water to obtain a liquid batter, sweetened; a spoonful of the batter was then fried with cooking oil in a pan with cupcake like depression, using a small artisanal ladle until golden for 2-5 min on each side to obtain the *massa* (Fig.3).

For the two sites, the four main steps of *massa* processing were the cleaning of raw material, milling, dough preparation and fermentation, and frying of the fermented dough. Thus, the key stages of fermentation occurred for the Zogona site during the soaking of the raw material during 12 to16 h and the settling of the dough which lasted 5 h. At the Bilbalogho site, fermentation occurred when the dough was set for 8 to 9 hours. Only millet was used for this study compared to Nkama and Malleshi (1998) study here other ingredients such as rice, groundnut or cowpea rice were added to a millet batter. In the same study trona, an impure evaporated mineral sodium sesquicarbonate salt was added to the preparation of massa dough to facilitate the leavening or swelling (sponginess) of *massa*.

Table 3. Physicochemical characteristics of samples from Bilbalogho site.

Sample	(%) Ash/DM	Dry matter (%)	Moisture (%)	pH	Titratable acidity (Lactic acid g/100 g DM)
Raw Material (Whole grains)	1.47± 0.04^{b}	90.66 ± 0.07^{a}	9.34 ± 0.07^{g}	6.4 ± 0.14^{a}	0.38 ± 0.00^{i}
Washed Millet grains	1.30± 0.02^{d}	77.85 ± 0.09^{b}	22.15 ± 0.10^{f}	6.16 ± 0.21^{a}	0.15 ± 0.01^{i}
Slightly dried millet grains	1.22±0.09^{-}	87.03 ± 0.09^{-}	12.97 ± 0.09^{-}	6.14 ± 0.06^{-}	0.16 ± 0.02
Flour	1.41±0.10^{c}	87.46 ± 1.08^{a}	12.54 ± 1.08^{g}	6.1 ± 0.00^{a}	0.20 ± 0.01^{h}
Sieved Flour	1.40±0.12^{-}	87.98 ± 1.00^{-}	12.19 ± 1.00^{-}	6.02 ± 0.06^{-}	0.18 ± 0.03
Dough before Fermentation	1.20±0.01^{f,g}	46.21 ± 1.25^{e,f}	53.79 ± 1.25^{b,c}	5.93 ± 0.12^{b}	1.29 ± 0.10^{a}
Fermented Dough	1.31±0.01^{f,gh}	41.75 ± 0.05^{g}	58.25 ± 0.05^{a}	4.17 ± 0.04^{b}	1.39 ± 0.01^{a}
Massa	0.83±0.01^{g,h}	60.61 ± 0.39^{d}	39.39 ± 0.40^{d}	4.86 ± 1.62^{a,b}	0.38 ± 0.02^{g}

DM: Dry matter; different letters in the same row indicate statistical significance ($p<0.05$).

Physico chemical characteristics of the samples from raw material to the finished products

Data indicated that the titratable acidity of the raw material (millet grains) was low and varied from 0.32 to 0.38 g lactic acid /100 g DM (Tables 2 and 3); the pH of the millet grains was 6.4 and this value is similar to those reported by DAO (2014) which were 6.5-6.6 for different varieties of millet grains from Burkina Faso According to DAO (2014), these values imply a very low oxidation of lipids in millet grains and signify a good preservation of millet grains used.

During the soaking step of millet grains in water at the Zogona site, water content of the millet grains increased (31.71 to 51.45%) and the pH decreased from 6.62 to 4.30 corresponding to an increase of the titratable acidity from 0.38 to 0.74 g lactic acid /100g (Table 1). Other data (Tou *et al.*, 2007) indicated similar results of lowered pH after soaking of millet grains for a minimum duration of 14 h in *ben-saalga* processing.

Table 4. Distribution of isolates according to production site.

Production Site	Bacilli	Coccobacilli	Cocci in tetrads	Cocci in pairs and chains	Total
Zogona (n=)	16	3	16	18	53
% Zogona Total	30.19	5.66	30.19	33.96	100.00
Bilbalogho (n=)	17	38	15	24	94
% Bilbalogho Total	18.09	40.43	15.96	25.53	100.00
Total (Zogona and Bilbalogho) (n=)	33	41	31	42	147
Total (Zogona and Bilbalogho) (%)	22.45	27.89	21.09	28.57	100

During the fermentation phase of the dough, the pH values do not vary significatively at the Zogona site (Table 2) but decreased from 5.93 to 4.17 at the Bilbalogho site (Table 3). The pH values at the end of fermentation of the dough (3.87 and 4.17 in both production sites) are similar to those reported by Songré-Ouattara *et al.* (2009) in a study on enriched millet dough for the preparation of *ben-saalga*, with pH values ranging from 3.9 to 4.19 at the end of fermentation.

Table 5. Distribution of EPS producing LAB based on capacity of production (confirmation test).

	Group 1 (EPS+)	Group 2 (EPS++)	Group 3 (EPS+++)	Total
Number of isolates	28	09	11	48
% of EPS+ isolates (n=48)	58.33	18.75	22.91	100
% referring to all isolates (n=186)	15.05	4.84	5.9	25.79

During the preparation of the dough to be fermented, the *massa* producer at Zogona site added fermented porridge called *ben-kida*. The acidity of *ben-kida* (pH 4.13) was lower than the acidity of the *ben-saalga* slurry (pH 6.06).

The *ben-kida* had been prepared the day before *massa* processing; the resulting low pH can justify the empirical use for backsloping technique, to accelerate the fermentation of the dough; the *ben-saalga* slurry was prepared and not cooled just before incorporation into the dough to be fermented. The temperature provided from the *ben-saalga* increased the temperature of the dough for improving the fermentation conditions and should contribute to accelerate the fermentation of the dough. During the process of *massa*, the pH decreased from raw material to the finished product. The pH value of washed millet grains at the Zogona site was 4.51 (Table 2) because the grains were soaked for 14 ± 2 h.

At the end of the fermentation, the pH value of the fermented dough at the two sites was 3.87 (Zogona site) and 4.17 (Bilbalogho site).

Statistical analyses show a significant difference between the pH values of the raw material and the dough at the end of fermentation ($p < 0.05$) at the two production sites.

The water content of the millet grains used as raw material and the flour obtained after milling ranged from 8.37 to 9.34 % and from 12.19 to 12.54% respectively.

Table 6. Results for screening of dextran producing strains on pearl millet sourdough; the strains characteristics and identification.

EPS Production	Strain code	Viscosity (pa /s)	pH à 24h	Taxonomic name	Origin	Amylase	Esculetin
EPS -	SD 0h[1]	0.20 ± 0.0 ^{g}	6.3 ± 0.0 ^{a}	-	nd	nd	nd
EPS -	Control lab 1[2]	0.20 ± 0.0 ^{g}	4.1 ± 0.1 ^{bc}	-	nd	nd	nd
EPS +++	lab 1	2.11 ± 0.1 ^{a}	4.2 ± 0.1 ^{b}	*W. cibaria/confusa*	Dough at the beginning of fermentation	++	-
EPS +++	lab 2	0.33 ± 0.0 ^{f}	4.1 ± 0.1 ^{bc}	*W. confusa*	Water for millin	+	+
EPS +++	lab 3	1.82 ± 0.3 ^{b}	4.2 ± 0.1 ^{bc}	*W. cibaria/confusa*	Dough at the beginning of fermentation	++	-
EPS +++	lab 4	0.33 ± 0.0 ^{f}	4.1 ± 0.0 ^{bc}	*W. cibaria/confusa*	Dough at the beginning of fermentation	+	-
EPS +++	lab 5	0.31 ± 0.0 ^{f}	4.1 ± 0.0 ^{bc}	*W. cibaria/confusa*	Dough at the beginning of fermentation	+	-

Updated from (Wang *et al.*, 2019).

[1] SD 0 h is prepared by mixing pearl millet flour with distilled water without the addition of starter, time 0 h means before fermentation.

[2]ControlLab1 cis prepared without sucrose addition.

These values are in accordance and lower compared to the maximal limit of 13% for moisture content of millet flour according to the CODEX standard (1989) for millet flour.

The ash content values ranged from 1.47 to 1.61 % DM for the millet grains (raw materials). The ash content of the flour obtained after grains milling is 1.40 % DM. This value is comparable to that obtained in millet flour (1.3% DM) by Nkama and Malleshi (1998).

At Zogona site, during the soaking of millet grains, the decrease in ash content from 1.47 to 1.32 % DM could be explained by the loss of matter through leaching. At the end of fermentation, the difference in the values of the ash content of the dough compared to the values obtained before fermentation of the dough are not significant. At Bilbalogho site, values of the dough ash content ranged from 1.20 to 1.31 after fermentation, but are not significant. Increase of ash content after fermentation have been explained by an incomplete utilization of minerals by fermenting organisms during their metabolism and reported by Ojokoh and Bello (2014) in their study on the effect of fermentation on the composition of a blend of flours.

Microbial characteristics

Microbial population associated to massa processing

A total of 52 samples including raw material (millet grains), intermediary products and finished products were collected and analyzed for AMB, enterobacteria, LAB, yeasts and molds counts. The results of microorganisms' growth during the different steps of *massa* process are shown in Figs 4 and 5 for Zogona site and in Figs 6 and 7 for Bilbalogho site.

At Zogona site, collected samples included whole millet grains, millet grains at the beginning and at the end of soaking, millet washed after soaking, dough obtained after grinding the grains, dough at the beginning and at the end of fermentation and *massa* the end product; intermediary samples included water used for soaking, water at the beginning of soaking, water at the end of soaking, water added at the mill for milling, *ben-kida* porridge and water for kneading/mixing. Microbial counts in the whole millet grains used as raw material, ranged from 4.89±0.07 to 5.48±0.02 log CFU.g^{-1} for AMB, from 3.36±0.04 to 4.27±0.02 log CFU.g^{-1} for enterobacteria, from 2.95±0.02 to 2.36±0.32 log CFU.g^{-1} for LAB and from 4.00±1.03 to 4.15±0.10 log CFU.g^{-1} for yeasts and molds (Fig. 4).

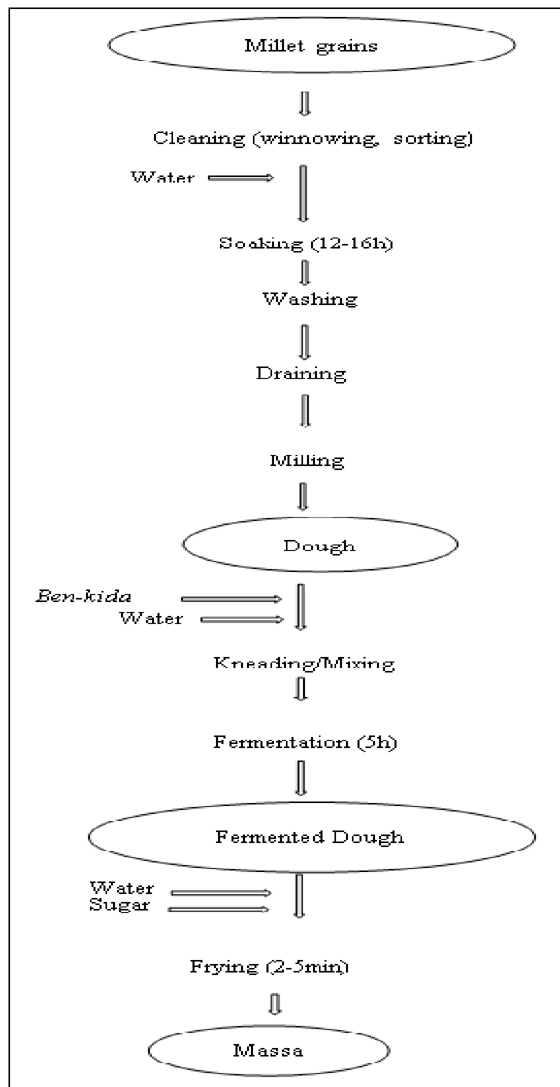

Fig. 1. Massa process at Zogona site.

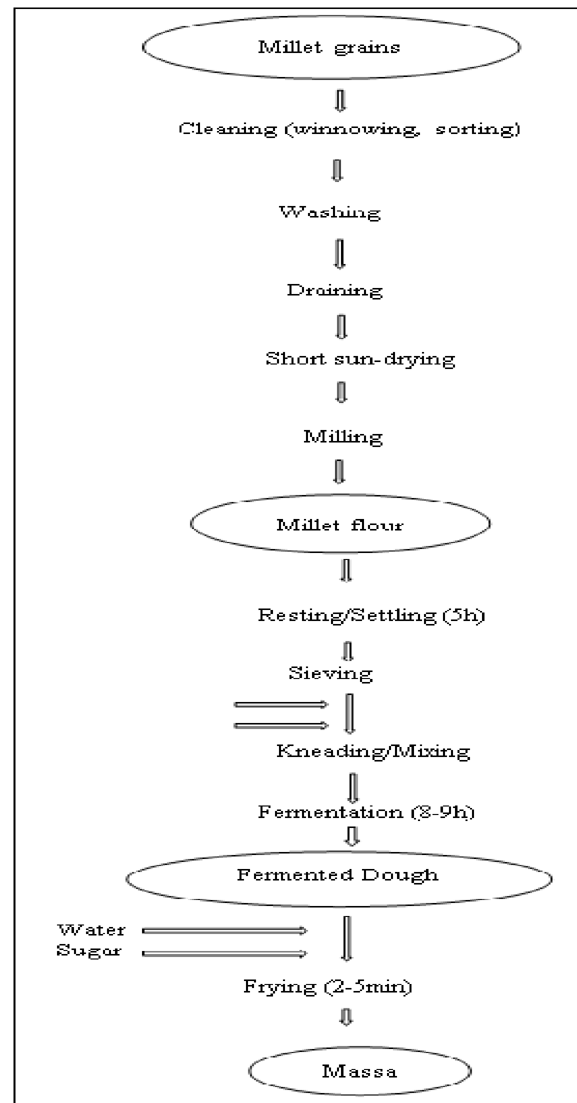

Fig. 2. Massa process at Bilbalogho site.

The microorganisms associated to *massa* process were dominated by aerobic mesophilic bacteria, enterobacteria, yeasts and molds and LAB. During the soaking, the microbial cell density increased, particularly for LAB counts (Fig.4) with mean values that ranged from 3.23 ±0.70 to 8.15±0.36 log CFU.g^{-1}(Fig.4) The two-fold increase of lactic acid bacteria during this step explain lactic acid fermentation and the decrease in pH observed during this step. The water used for soaking was not a source of contamination because its initial bacterial cell density was <1 log CFU.mL^{-1}(Fig.5) At the end of soaking, LAB cell density in water increased consequently from3.47±1.11 to 8.38±0.57 log CFU.mL^{-1}(Fig.5) The load of lactic acid bacteria in the millet grains increased that of the water from less than 1 log CFU to 3.47 log CFU.

After milling the grains to obtain a dough, the microbial load of the dough was higher compared to that of the soaked grains. Especially for enterobacteria counts with mean values ranging from 4.27±0.26 for washed millet (step prior milling) to 5.04±0.11log CFU.g^{-1} for the dough (obtained after milling). Yeasts and molds counts also increased with mean values ranging from 2.23±0.23 to 6.58±1.18 log CFU.g^{-1}. This growth could be explained by the favorable environmental conditions such as open air, availability of water and nutrients.In fact, the water used at the mill had a microbial cell density ranging from 1.36±0.26 to 4.58 ± 0.01 log CFU.g^{-1} for its AMB and from 1.00 to 1.53±0.04 log CFU.g^{-1} for lactic acid bacteria (Fig.5); it probably contributed to the increase in the microbial load of the dough.

Fig. 3. Frying the fermented dough at Bilbalogho site.

The porridge added to the dough during its preparation for fermentation had a microbial flora dominated by the presence of LAB and yeasts and molds.

The respective mean values ranged from 4.94±0.01 to 5.99±0.17log CFU.g^{-1}for LAB, and 4.63±0.52 to 6.15±0.04 log CFU.g^{-1}for yeasts and molds (Fig.5). The interest of using this porridge for backsloping could be justified by an empirical knowledge backed by a scientifically proven LAB count.

At the end of dough fermentation, mean values of LAB counts increased from 7.02±1.56 to 9.25±0.57 log CFU.g^{-1} and mean values of enterobacteria counts decreased from 4.21±0.89 to 1.88±1.11 log CFU.g^{-1}.

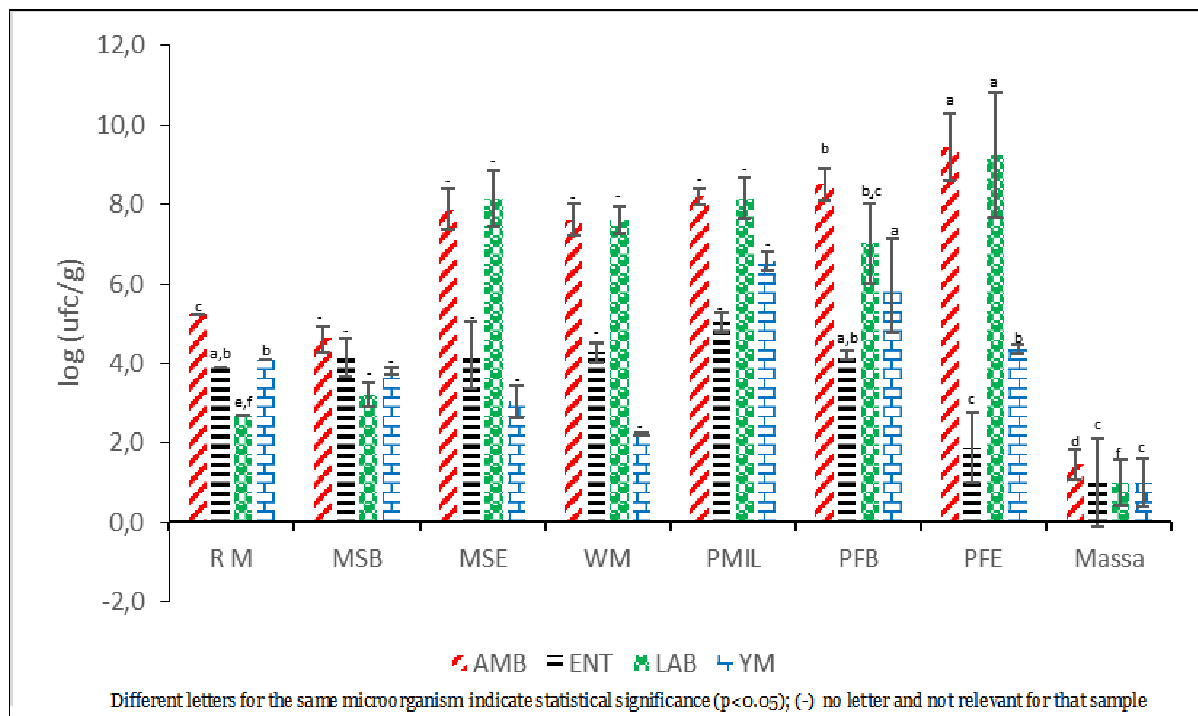

Fig. 4. Microorganism's counts in the samples collected during massa processing at Zogona site.

RM: raw material, MSB: Millet soaking (begin), MSE: Millet soaking (end), WM: washed millet, PMIL: dough from milling, PFB: dough fermentation begin, PFE: dough fermentation end;

AMB: aerobic mesophilic bacteria; ENT: *Enterobacteria*; LAB: lactic acid bacteria; YM: yeasts and molds.

*Mass*a samples were collected immediately after frying and were characterized by a low microbial cell count with mean values ranging from 1.00±0.00 to 2.36±0.26 log CFU.g^{-1} for AMB and less than 1.00±0.00 log CFU.g^{-1} for enterobacteria, LAB, yeasts and molds. This low microbial load could be explained by the action of heat during frying on the microorganisms present in fermented dough. *Massa* is a product that is not preserved; the low bacterial load demonstrates a good hygienic quality of the product just after frying The hygienic quality of the final product must be preserved during handling for

sale to consumers; so they can fully benefit from the product quality and preserve their health.

Even though the general trends were similar for microbial analyses, results for each production were different; this difference is explained by the fact that the process is artisanal and not controlled. At Zogona site, the fermentation of the dough is confirmed by the development of LAB and the decrease of the pH; the soaking step is also an important fermentation step during which LAB growth is enhanced; these fermentations were spontaneous and non-controlled.

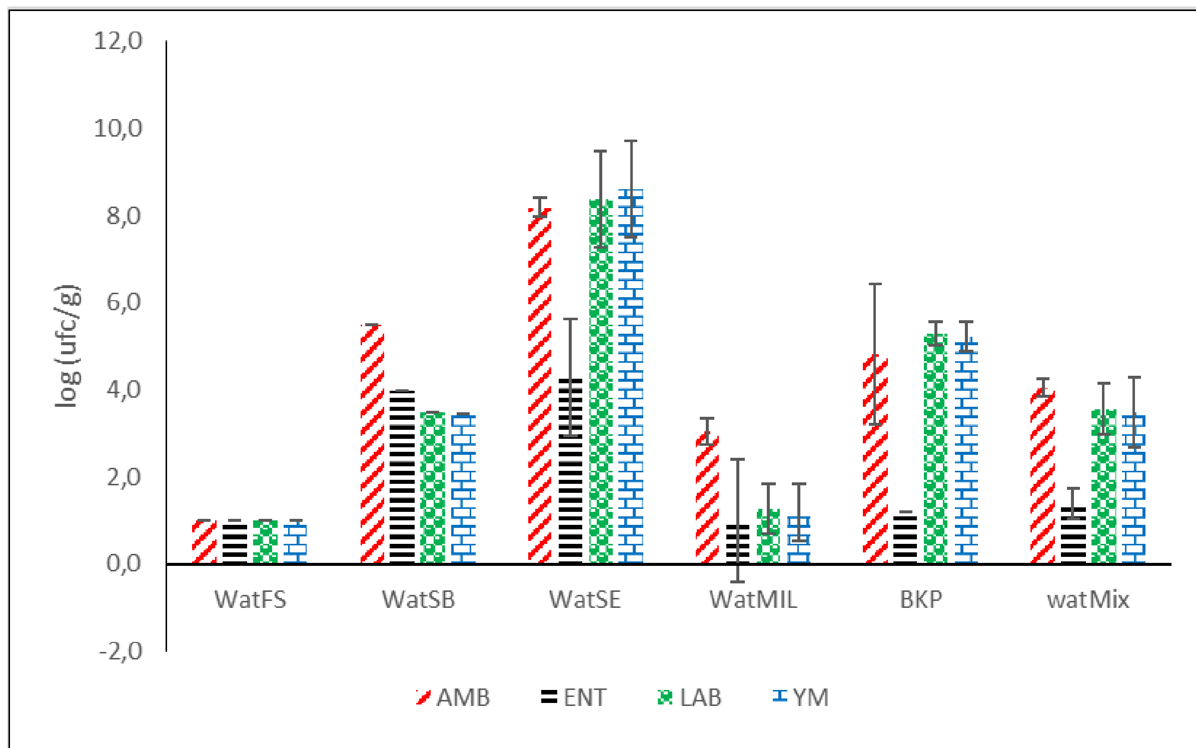

Fig. 5. Microorganisms counts from water and porridge samples at Zogona site.
WatFS: water used for soaking, WatSB: water soaking beginning, watSE: Water soaking End, WatMIL: water for milling, BKP: *ben-kida* porridge, watMix: water for mixing; AMB: aerobic mesophilic bacteria; ENT: *Enterobacteriaceae*; LAB: lactic acid bacteria; YM: yeasts molds.

During soaking, fermentation can be attributed to the original microbiota of the millet grains. Similarly, in a previous study on fermented grains, Sawadogo-Lingani (2010) concluded that bacteria mainly responsible for the natural fermentation of sorghum for malt production originated from the grains microbiota.

For the dough at the end of fermentation, mean values of LAB counts ranged from 8.85±0.02 to 9.91±0.00 log CFU.g^{-1} and were higher compared to the value of 7.00 log CFU.g^{-1} obtained by Tou(2007) at the end of fermentation of a millet dough used to prepare *ben-saalga* porridge. The decrease in the number of enterobacteria at the end of fermentation of the dough could be attributed to the growth of LAB producing acids, which will consequently decrease the pH and inhibit the development of non-desirable microorganisms (Adams and Moss, 2008). Tou (2007) identified the following products at the end of fermentation: ethanol, acetic and lactic acids with ethanol as the main products for the end of soaking and lactic acid as the main product at the end of fermentation of liquid doughs made for the preparation of millet-based *ben-saalga* porridges.

At Bilbalogho site, the samples represented in Fig.6 included millet grains (raw material), washed millet, millet after a short drying, flour obtained after milling, sieved flour, dough at the beginning of

fermentation, dough at the end of fermentation, and the finished product obtained after frying(*massa*). Secondary samples included millet wash water, kneading water and *ben-saalga* porridge.

The microbial cell density of the raw material in Bilbalogho site ranged from 4.93±0.01 to 5.08±0.05 log CFU.g^{-1}for AMB, 1.80±0.20 to 4.15±0.04 log CFU.g^{-1}for enterobacteria, 4.14±0.09 to 5.11±0.05 log CFU.g^{-1}for LAB and 3.45±0.00 to 4.95 ±0.00log CFU.g^{-1} for yeasts and molds. These results were close to those of Zogona site and confirmed the diversified microbiota of the millet grains (Fig. 5).

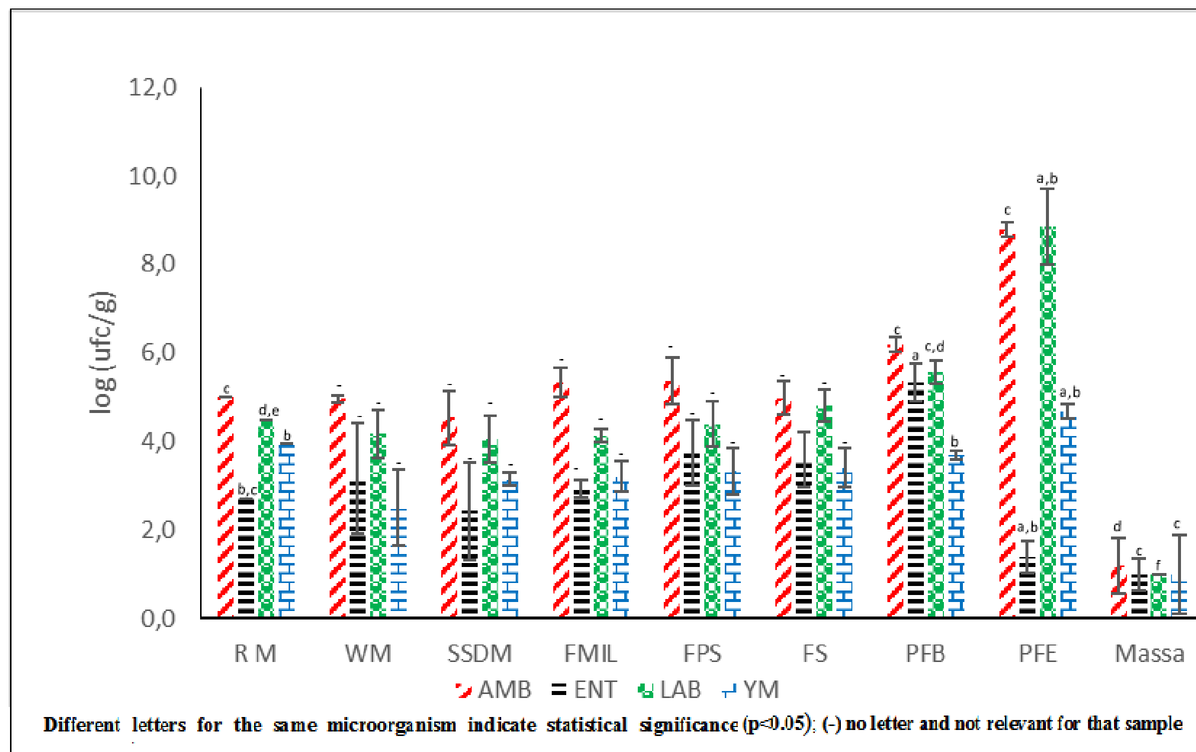

Fig. 6. Enumeration of microorganisms of main samples from Bilbalogho site.
R M: raw material, WM: washed millet, SSDM: short sun-dried millet, FMIL: flour from milling, FPS: flour prior sieving, FS: flour sieved, PFB: dough fermentation begins, PFE: dough fermentation end, AMB: aerobic mesophilic bacteria; ENT: *Enterobacteria*; LAB: lactic acid bacteria; YM: yeasts molds.

Washing the raw material reduced the population of yeasts and molds, but *enterobacteria* counts increased with mean values ranging from 2.72±1.26 to 3.17±1.09 log CFU.g^{-1}. The sources of this contamination could be related to the production environment (personnel, equipment, air and so on).

The flour obtained after milling of the washed and dried millet grains showed high load for aerobic mesophilic bacteria (AMB) with a mean value of 5.34±0.52 log CFU.g^{-1}that was reduced to 4.99±0.16 log CFU.g^{-1} after sieving. The flora was dominated by LAB and yeasts. These results were similar to those of Tou (2007), who found in millet flour (unfermented) a flora dominated by the presence of LAB and yeasts.

The *ben-saalga* porridge added for the preparation of the dough to be fermented showed low bacterial load (Fig. 6) and was mainly composed of AMB with values ranging from 1.90±0.14 and 3.29±0. log CFU.g^{-1}. This porridge was prepared just before being added to the dough, which could explain the reduced microbial cell density; the action of heat inhibits many heat-sensitive microorganisms.

At the end of fermentation, the microbiota of the dough was dominated by LAB with a mean cell density value of 8.85±0.01log CFU.g^{-1}, and a

decreased count of enterobacteria with values of 1.40±0.35 log CFU.g^{-1}.LAB counts increased by a factor of 1.5 logarithmic units; these values are lower than those found by Songré (2009). In that study, from the fermentation of a dough composed of a mixture of cereals and legumes for the preparation of a *ben-saalga*-type porridge, an increase of 2 logarithmic units for LAB was noted at the end of fermentation. These results could be explained by the short duration of the fermentation stages.

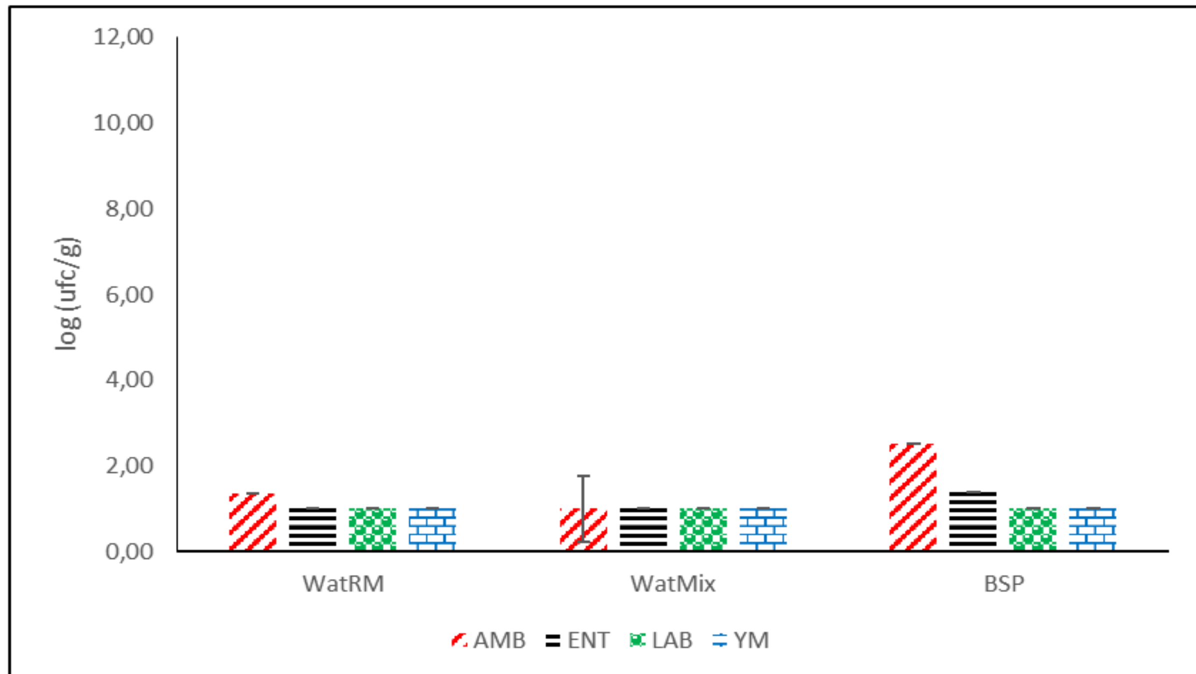

Fig. 7. Microbiological enumeration of water and porridge samples from Bilbalogho site.
WatRM: water for raw material washing, WatMix: water for mixing, BSP: bensalga porridge; AMB: aerobic mesophilic bacteria; ENT: *Enterobacteria*; LAB: lactic acid bacteria; YM: yeasts molds.

Massa, the end product of the process was characterized by a low microbial count lower than 1.00 log CFU.g^{-1}for AMB, enterobacteria, LAB and yeasts and molds. Based on these results, *massa* is a product with a satisfactory hygienic quality. These results are similar to those of Zogona production site. These results could be explained by the sampling by the fact that samples were collected just after frying. In comparison, the study of Badau (2018) on "masa" microbial quality revealed coliform counts higher than the values found in our study. Therefore, in the Badau (2018) study, pathogenic microorganisms like *Shigella spp*, *Salmonella spp*, *Pseudomonas sp*, *E. coli* and *Staphylococcus* were identified in those samples. Consequently, it is necessary to maintain hygienic quality during the sale and storage of *massa.* For both production site, water is common among intermediary samples which were for Zogona site: water for soaking, water at the beginning of soaking, water at the end of soaking, water used by the miller, *ben-kida* porridge. At Bilbalogho site, the intermediary samples were: water for raw material washing, water for dough mixing and *ben-salga* porridge. Pertaining to the probability of being a source of contamination for the process, the quality of water is important. Referring to Figs. 5 and 7, the water used by Zogona *massa* producer is of better microbial quality in comparison to the water used by the miller to mill the grains into dough. Indeed, the water was collected from the tap faucet just before being used for soaking. However, at the mill, the water used had been stored and the high microbial concentration is due to the poor storage conditions.

Similarly, to the water used at the mill, the water used for kneading/mixing had probably also been stored in poor conditions which explains the resulting high microbial cell count.

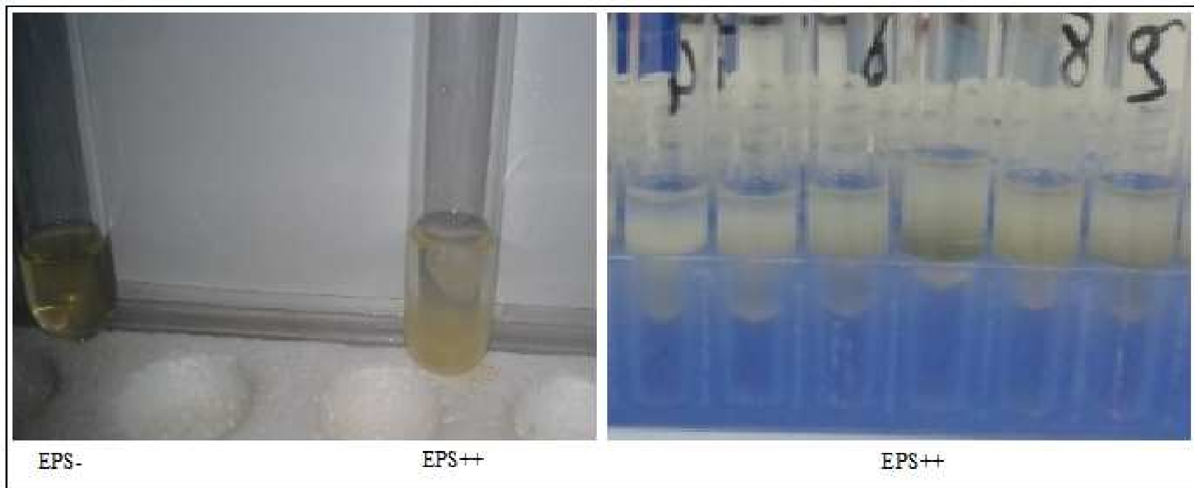

Fig. 8. EPS Production 1st step.

Compared to Zogona production site, the water used at Bilbalogho site showed a better quality with a low microbial cell density. The water used to wash the raw material and knead the millet flour into dough was collected from the tap faucet just before using. These results confirm that the water from the national utility provider ONEA is of good microbiological quality.

LAB isolates characteristics

A total of 600 colonies were collected from the mMRS medium and characterized by biochemical tests. From all the isolates collected, 31% (n=186) were Gram +, catalase -, oxidase- and non-motile bacteria thus supposedly lactic acid isolates. Among these, 147 isolates representing 79.03% of presumptive LAB were tested to achieve preliminary gender characterization.

The distribution of these isolates according to the production site is presented in Table 4. Based on the production site, the characterized isolates consist of 36.0% from Zogona production site (n = 53) and 64.0% from Bilbalogho site (n = 94).

Fig. 9. EPS Production 2nd step (Confirmation).

The distribution of isolates characterized by morphology (Table 4) reveals that they were mainly cocci in pair and chains at 28.57% (n = 42), short rods (coccobacilli)at 27.89% (n = 41), and rods (bacilli) at 22.45% (n = 33) and cocci in tetrads at 21.09% (n=31). In total cocci bacteria were the majority and represent 49.65% (n=73) of all isolates. Referring to production sites (Table 4), 23.12% (n = 34) of the isolates were mainly cocci bacteria from Zogona and accounted 64.15% of Zogona isolates. Among the

isolates of Bilbalogho cocci bacteria represented 26.53 % (n = 39) of all isolates and accounted for 41.5% of isolates from Bilbalogho.

Presumptive identification of the LAB isolates at genus level

Referring to the characteristics presented in table 1 and the tests performed, the LAB isolates were classified into presumptive genuses. Among the heterofermentative bacilli, growth at 45°Cwasa criterion to distinguish bacilli of the genus *Lactobacillus* from bacilli of the genus *Carnobacterium* (Table 1). Coccobacilli which fermented glucose with gas production were classified as *Lactobacillus* or *Leuconostoc*; those which grew at 45°C were classified as *Lactobacillus*. In this group, the isolates that didn't grew at pH 4 were counted as belonging to *Leuconostoc*. Homofermentative coccobacilli were classified in the genus *Streptococcus* (Table 1); homo/heterofermentative coccobacilli were presumed *Lactobacillus*.

Isolates of cocci morphology and grouped in tetrads could belong to the genus Aerococcus, *Pediococcus*, or *Tetragenococcus*. In this group, isolates belonging to the genus *Pediococcus* were distinguished by growth at 45°C. Homofermentative isolates of cocci morphology grouped in pairs and chains were classified as *Streptococcus* because they were distinguished from the genus *Lactococcus* based on their positive growth at 45°C. Among heterofermentative isolates in this group, those that do not grow at 45°C were classified as *Leuconostoc* or *Weissella*. Heterofermentative cocci grouped in pairs and chains isolates growing at 45°C were not classified.Based on their characteristics, the LAB isolates (n=108) collected from massa process at the Zogona and Bilbalogho sites were classified into the following presumptive genuses: *Lactobacillus* (16.39%; n=20), *Lactobacillus/Weissella* (6.56%; n=8), *Leuconostoc* (18.85%; n=23), *Streptococcus* (31.97%; n=39), *Pediococcus* (8.20%; n=10), *Aerococcus/Pediococcus/Tetragenococcus* (5.74%; n=7), *Streptococcus / Lactococcus* (0.82 %; n=1). The microbiota of tropical fermented products such as millets and other non-wheat cereal grains have been found to be dominated by LAB from *Lactobacillus* genus and a variety of species belonging to *Leuconostoc* and *Weissela* genuses (Coda *et al.*, 2014; Tapsoba, 2018).

EPS producing isolates and their identification

Out of a total of 600 isolates collected from *massa* production, 186 were presumptive LAB among which 48 isolates (25.79%) presented an EPS production capacity (Table 5). The EPS producing isolates were mainly found from samples of Zogona and represent 81.25% (n=39) of EPS producing isolates. They were mainly collected from samples of dough before fermentation that accounts for 25% of EPS producing isolates (n=12). The back-slopping process utilized to mix the dough for fermentation added to the conditions at the mill on Zogona process site could explain these results. The 48 isolates were classified into 3 groups based on EPS production capacity (Table 5) as shown in Figs 8 and 9. Among the eleven EPS +++ isolates (Table 5), five were selected for their EPS producing capacity in millet sourdough. The results of viscosity test with these five isolates showed that millet sourdough inoculated with lab1 presented the highest viscosity (Wang *et al.*, 2019).All five isolates were collected from Zogona *massa* production site, their characteristics are presented in Table 6. They fermented glucose in MRS broth with Durham Bell with mixed acids as by products confirmed with the red methyl test. The isolates with the highest amylase degradation capacity, presented the higher viscosity in results presented from Wang *et al.*, 2019. From these results, the isolateLab1 was identified as *Weissella. confusa* at *98%* (Wang *et al.*, 2019). The isolate Lab 1 has been collected at the Zogona *massa* production site from the dough before fermentation.

Utilizations of EPS on massa process

The starter culture was able to produce EPS in the sourdough. However, the presence of EPS had a negative effect on frying. The diluted dough couldn't be fried due to its stickiness to the frying pan. The structure of the EPS which is slimy and gel type

impacted negatively massa. Most application of EPS in food science can be found in bakery and the beverage industry. Further work should be pursued to optimize and develop those type of products.

Conclusion

The study allowed to establish the process diagrams of whole millet grains *massa*, a fermented pancake type product from Burkina Faso. These results suggest that there is a diversity of microorganisms involved in the *massa* production process and that lactic acid bacteria are responsible for *massa* dough fermentation. After frying the fermented dough to obtain *massa*, the microbiota considerably decreased. The Heat had an impact on these microorganisms. Presumptive lactic acid bacterial isolates were collected and characterized.

They were mainly composed of cocci, coccobacilli (short rods) and bacilli (rods) with presumptive genus of *Lactobacillus, Weissella, Leuconostoc, Streptococcus, Pediococcus, Aerococcus/Tetragenococcus, Lactococcus*. The screening of EPS production from these LAB isolates resulted in the selection of one *Weissella confusa* strain Lab 1 with the highest exopolysaccharides (EPS) producing capacity in millet sourdough. Even though the production of EPS in the preparation of massa sourdough had a negative impact on the product, this EPS had shown potential and had been used as a hydrocolloid for its potential to improve the sensory quality of food products, such as breads.

Declaration of Conflict of interest

Authors declare that there is no conflict of interest.

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