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Full Length Research Paper

Improvement of nutritional, sanitary and organoleptic qualities of liquid zoom-koom and instant flour zoom-koom using *Lactobacillus fermentum* starter culture

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Traditional fermented beverages occupy an important place in the diet of populations in Burkina Faso. Zoom-koom in particular remains one of the most consumed cereal-based drinks in cities like Ouagadougou. The drink zoom-koom is consumed primarily in the liquid form and to short scale in the reconstituted form called instant flour zoom-koom. Being able to diversify zoom-koom taste and aroma and to improve the nutritional and hygienic quality would definitely add value to this product. For sensorial analysis, 04 formulations of liquid zoom-koom and instant flour zoom-koom were realized and evalued by a panel of tasters. The best formulation of each type of zoom-koom was produced using *Lactobacillus fermentum* strains *SF9.5* and *SF6.2* as starter culture. Then, the microbiological (aerobic mesophilic bacteria, lactic acid bacteria, coliforms, yeasts and molds), physico-chemical (pH, acidity, proteins, sugar, lipids, iron, magnesium, calcium) and sensory parameters of each product were determined. The results showed a marked improvement in protein content (25.83-28.41%) for instant flour zoom-koom, in reducing sugars for liquid zoom-koom (32.1-36.9%) and for instant flour zoom-koom (29.88-47.43%), in calcium (39-43 mg for liquid zoom-koom), in magnesium (59-65 mg for liquid zoom-koom), in iron (2.37-2.50 mg and 3.0-3.08 mg respectively for liquid zoom-koom and instant flour zoom-koom). A reduction in total coliforms from 1.3×10^4 to 4.0×10^1 cfu/ml for the liquid zoom-koom and from 4.3×10^4 to 6.8×10^3 cfu/ml for the instant flour zoom-koom was observed. In addition, a reduction in mold growth ranging from 1.3×10^4 to 4.0×10^1 cfu/ml for the liquid zoom-koom and from 1.3×10^4 to 7.0×10^3 cfu/ml for instant flour zoom-koom was observed. The zoom-koom samples using *L. fermentum* starter cultures were well appreciated by at least 80% of the tasters.

Key words: Beverage, zoom-koom, *Lactobacillus fermentum*, starter cultures, quality.

INTRODUCTION

Traditional foods including drinks play a major role in African societies. In West Africa especially in Burkina Faso, traditional cereal-based (sorghum, maize or millet) beverages participate daily to the dietary habits of

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Table 1. Formulations of liquid zoom-koom (L.Z.K).

Samples	Formulations			
	Liquid zoom-koom with lemon (LZK1)	Liquid zoom-koom with Milk(LZK2)	Liquid zoom-koom with tamarind (LZK3)	Liquid zoom-koom with baobab pulp (LZK4)
Millet / g	900	900	900	900
Maize / g	0	0	0	0
Pulp of tamarind / g	0	0	600	0
Pulp of baobab / g	0	500	0	600
Dried Milk / g	0	400	0	0
Ginger-Mint / g	800	800	800	800
Jus of lemon / ml	500	0	0	0
Sugar / g	1000	1000	1000	1000

populations (Barro et al., 2003; Tankoano et al, 2017). Fermentation has long been one of the methods of processing and preserving traditional foods (Yao et al., 2009). Lactic acid bacteria, yeasts and molds were identified as the main microorganisms observed during food fermentation (Yao et al, 2009). Lactic fermentation is known as a biochemical process which can enhance aroma and texture (Tapsoba et al., 2017c). Previous studies on controlled lactic fermentation of traditional zoom-koom-like beverages such as kunun-zaki in Nigeria (Gaffa et al., 2002; Amusa et al., 2009; Agarry et al., 2010) showed at the end of the technological process, a marked improvement in the nutritional, hygienic and organoleptic qualities of kunun-zaki. The utilization of starter cultures in the processing can be a solution for the improvement of nutritionnal, organoleptic and sanitary quality of indigenous foods and beverage such as zoom-koom.

The most widely consumed traditional drinks in Burkina Faso are dolo, bandji and zoom-koom (Icard-Vernière et al., 2010). Zoom-koom is a traditional millet-based drink derived from millet or sorghum grains by lactic fermentation (Soma, 2014). It is sold and consumed in all parts of Burkina Faso by urban as well as rural people. Tapsoba et al. (2017a) showed that fermented millet zoom-koom is better than fermented sorghum zoom-koom, but unfermented sorghum zoom-koom is better than unfermented millet zoom-koom in terms of microbiological quality.

However, zoom-koom process is spontaneous, uncontrolled and usually made with varied fermentation times and temperatures, resulting in products inconsistent in quality attributes (Soma et al., 2017). In order to avoid growth of undesired microorganisms including pathogenic and spoilage microorganisms, starter cultures can be used. By the use of starter cultures, it is possible to control fermentation of such condiments, avoiding growth of pathogenic and spoilage microorganisms, leading to a product of consistent taste and quality, as well as improved marketability (Ouoba et al., 2008). In fact, Soma (2014) showed throughout a control production of millet zoomkoom using *Lactobacillus fermentum* strain as starter, that the lactic fermentation allowed the reduction of enterobacteria counts and kept safe the final product. Moreover, Tapsoba et al. (2017b) characterized and identified the selected LAB isolates, which could be used as starters' cultures to improve microbiological quality and the texture of zoom-koom and then they found that *Lactobacillus* strains were the dominant bacteria involved in the fermentation of zoom-koom. The present study consisted to realize the formulation of liquid zoom-koom and instant flour zoom-koom using starter cultures in order to determine their impact on the nutritional, sanitary and organoleptic quality of these beverages.

MATERIALS AND METHODS

Traditional production of liquid zoom-koom and instant flour zoom-koom were followed in order to establish the diagram production. Then 04 formulations (Tables 1 and 2) for each type of zoom-koom were prepared and submitted for sensorial, biochemical and microbiological analyzes. The best formulation of each type was chose for control fermentation using *L. fermentum* strain as starter. The final product obtained by controlled fermentation was also analyzed.

Traditional production of liquid zoom-koom and instant flour zoom-koom

Study area

The study took place in Ouagadougou, Burkina Faso. 02 artisanal producers (one producer of liquid zoom-koom located at Zogona district and another producer of instant flour zoom-koom located at Pissy district) have been selected for the study. In each production site, 03 productions were followed up during 03 weeks for the establishment of diagram production. The biological material required for zoom-koom production consists mainly of millet (*Pennisetum glaucum*) grains and ingredients such as tamarind (*Tamarindus indica*), mint (*Mentha spicata* L), ginger (*Zingiber officinale*) and sugar (Soma, 2014).

Traditional production of liquid zoom-koom

From the follow-ups, the liquid zoom-koom (Figure 1) was obtained

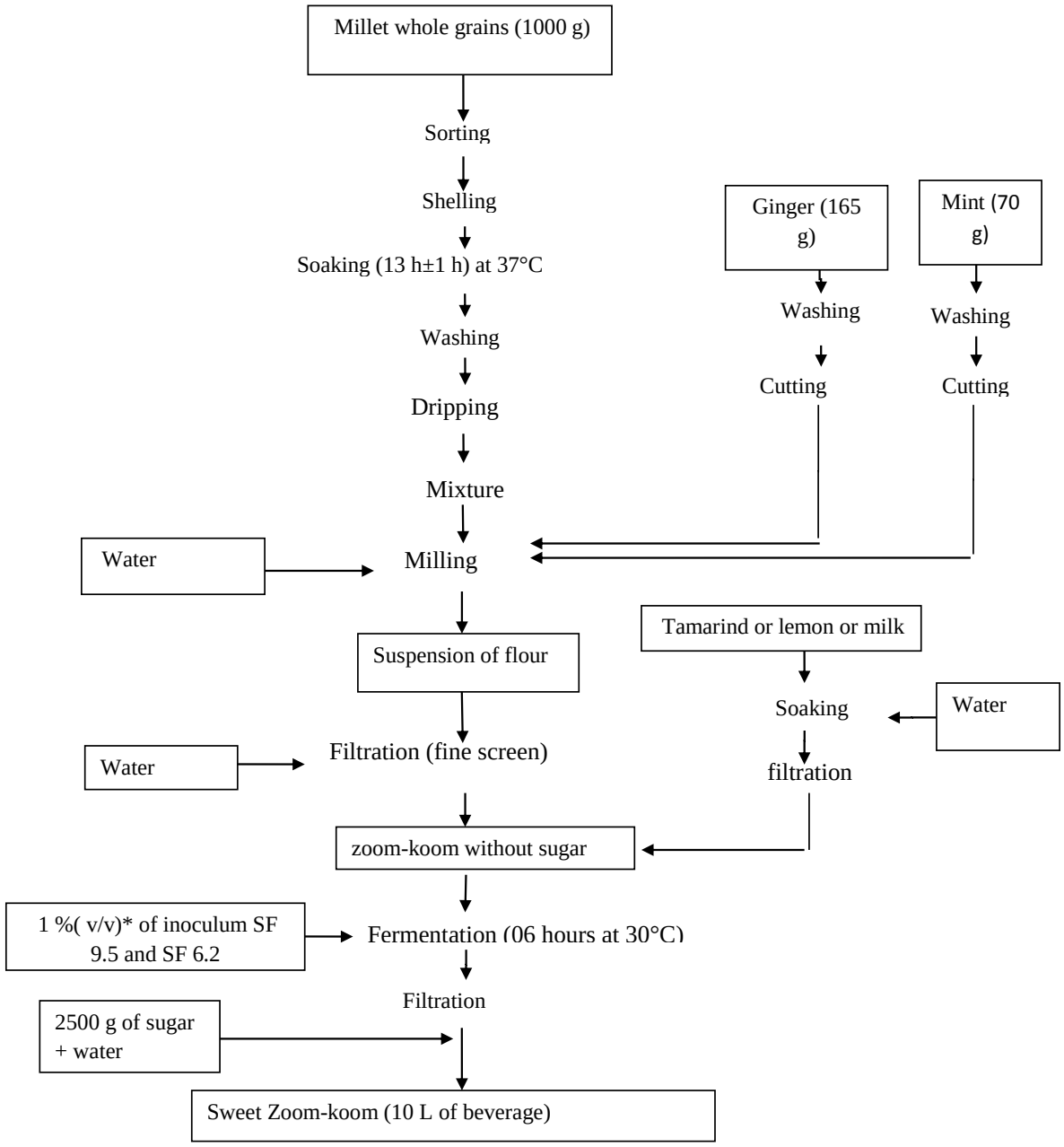

Figure 1. Production diagram of the liquid zoom-koom in Zogona site.
(*): Step additionally made from the zoom-koom liquid produced using *L. fermentum* SF 6.2 and SF 9.5 inoculum.

Table 2. Formulations of instant flour zoom-koom (IFZK).

Samples	IFZK with milk	IFZK with baobab pulp	IFZK with tamarind	Traditional IFZK
Millet / g	2000	2000	2000	2000
Maize / g	0	150	300	0
Pulp of baobab / g	0	300	0	0
Pulp of tamarind / g	0	0	600	0
Ginger–Mint / g	760	760	760	760
Dried Milk / g	150	0	0	0
Sugar / g	600	600	600	600

after 10 processing steps (Icard-Vernière et al., 2010; Soma, 2014; Tapsoba et al., 2017) which are: sorting the millet grains, peeling, soaking during 13 h, washing, draining the grains, mixing with ginger (6 g/ 100 g) and mint (3 g/ 100 g), milling, filtration and sweetening (2500 g/ 5 L).

Traditional production of instant flour zoom-koom

The instant flour zoom-koom was obtained after 14 steps from the raw material to the final product (Soma, 2014): sorting the millet grains, peeling the grains, soaking, washing, draining, mixing with ginger and mint, milling, filtration, first fermentation for 06 h at 37°C, second fermentation for 12 h at 37°C, squeezing of the pellet, drying of the pellet at 40°C for 09 h, sugaring the dried paste and finally sieving.

Zoom-koom formulations

The zoom-koom formulations took place in our pilot plan named Technopole.

Liquid zoom-koom formulations

A total of 04 liquid zoom-koom formulations were prepared according to the artisanal production diagram (Figure 1). The formulations are distinguished by using separately lemon, baobab pulp, milk and tamarind. The formulations of liquid zoom-koom were summarized in Table 1.

Instant flour zoom-koom formulations

A total of 04 instant flour zoom-koom formulations were prepared according to the production diagram developed. 03 formulations were based separately on baobab pulp, tamarind and milk and the last formulation contained any baobab pulp, any tamarind and any milk. The composition of each formulation is summarized in Table 2.

Controlled fermentation in zoom-koom production *Origin of L. fermentum strains used as starter culture*

L. fermentum strains (SF6.2 and SF9.5) used as starter culture in zoom-koom production, were isolated from dolo and pito (sorghum beer) (Sawadogo/Lingani et al., 2007;
2008). The 2 strains were chose from 45 efficient selected strains based on their acidifying capacity, their antimicrobial activity, their capacity to produce amylases and their ability to produce exocellular polysaccharides (Sawadogo-Lingani et al., 2008).

Preparation of the inocula of L. fermentum SF9.5 and SF6.2

Each *L. fermentum* strain stored in glycerol at -80°C, were subcultured on MRS Agar (Liofilchem, Italy), incubated for 48 h at 37°C. The colony was transferred into 10 ml MRS broth in tube, incubated for 24 h at 37°C. Then 0.1 ml of culture broth was transferred in 10 ml MRS broth and incubated for 16-18 h at 37°C. For each strain, the culture broth was centrifuged at 8000 g for 5 min. The supernatant of each tube was removed and the pellet was washed twice in 1 ml of sterile saline (8.5 g/L NaCl and 1.5 g/L bactopeptone (Difco, France), pH 7.0). After stirring, the suspension of cells (inoculum), the concentration of viable cells was determined as described by Sawadogo-Lingani et al. (2008).

Production of liquid zoom-koom and instant flour zoom-koom by controlled fermentation using L. fermentum strains SF9.5 and SF 6.2

Liquid zoom-koom and instant flour zoom-koom were produced according to the method described in Figures 1 and 2 respectively. The inoculum of *L. fermentum* was used at a rate of 1% (V / V) as described by Sawadogo et al. (2008). 100 ml of inoculum, composed of 50 ml of *L. fermentum* SF6.2 and 50 ml of *L. fermentum* SF9.5, were used to inoculate separately 10 L of liquid zoom-koom and 10 L of instant flour zoom-koom to obtain a final rate of 106 cells/ml. The mixtures were incubated at 37°C.

Sampling

From the 04 formulations prepared for each type of zoom-koom, the samples were collected for biochemical (pH and acidity), microbiological (aerobic mesophilis bacteria, lactic acid bacteria, coliforms, yeasts and molds) and sensory analyzes (grading test). Sampling was also done during controlled fermentation for biochemical (pH, acidity, protein, fat, total and reducing sugar, ashes, degree brix, mineral contents), microbiological (aerobic mesophilic bacteria, lactic acid bacteria, coliforms, yeasts and molds) and sensory analyzes (difference and profile tests). Moreover during zoom-koom formulations and controlled fermentation assays, sampling was done on the raw material (millet), technological adjuvants (maize, tamarind pulp, baobab pulp, mint, ginger, sugar), millet grains during soaking (beginning and end), the liquid zoom-koom and instant flour zoom-koom.

Biochemical analyzes of liquid zoom-koom and instant-flour zoom-koom

Measurement of pH and titratable acidity

The pH of the samples was measured using an electronic pH meter (Model HI 8520, Hanna Instrument, Singapore). For the solid samples, 10 g of product were mixed with 20 ml of distilled water before pH measurement. For liquid samples, pH was measured directly in the 10 ml of beverage (Sawadogo-Lingani et al., 2007). For determination of the titratable acidity, 5 g or 5 ml of sample suspended in 50 ml ethanol (90%) were centrifuged for 5 min at 3500 g. From the supernatant, 10 ml were transferred in a 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 an indicator (Sawadogo-Lingani et al., 2007). The titratable acidity (g of lactic acid per 100 ml or g of sample) was calculated according to Amoa-Awua et al. (1996).

Determination of protein content

Crude proteins contents were determined from the total nitrogen assay according to the Kjeldahl method NF V 03-50 (1970). The procedure comprises a mineralization, a distillation and a titration by sulfuric acid.

Determination of fat content

The fat content of the samples was determined by Soxhlet extraction according to the international standard (ISO-659, 1998). The extraction was carried out with hexane. The fat content was determined by weighing after evaporation of the hexane by distillation.

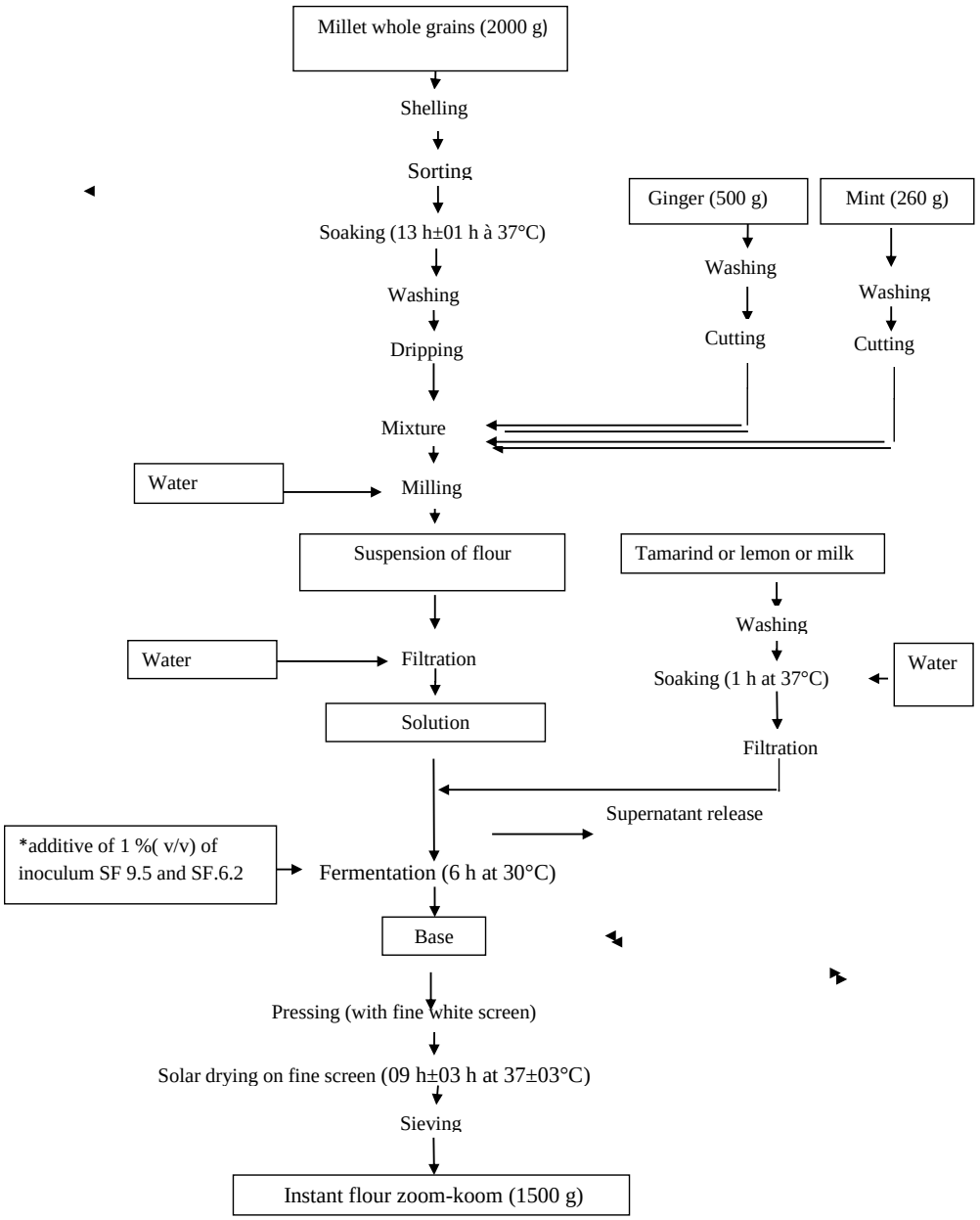

Figure 2. Production diagram of instant flour zoom-koom in Pissy site.

Determination of total and reducing sugar contents

Total sugar content has been estimated according to Montreuil and Spik (1963). 0.2 g of each sample was weighed and diluted in a flask containing 200 ml of distilled water. Then, 2 ml of orcinol solution and 7 ml of sulphuric acid solution 60% were added to 1 ml of the obtained solution. The mixtures were boiled for 20 min and placed in a dark place during 45 min. The reading of the optic density was done by a spectrophotometer (PG Instruments, England) at 510 nm. The standardization curve has been achieved using glucose (0.5 mg/ml) as reference. For reducing sugars, the quantification was done according to the method of Bernfeld (1955). The previous extract was introduced into a test tube; 0.5 ml of distilled water and 0.5 ml of DNS (Di-Nitro Sulfate) solution were added. The mixture was heated in a water bath at 90°C for 5 min. After cooling, 2 ml of distilled water were added and then the absorbance of the solution was read using a spectrophotometer (PG Instruments, England) at 490 nm. The standardization curve has been achieved using glucose (0.5 mg/ml) as reference.

Ashes contents determination

The ashes content was determined according to the standard V03-760 (1981). In three crucibles, 3 g of zoom-koom samples were placed in each one. The crucibles were submitted to a mineralization in the oven (Nabertherm) at 650°C during the night (16 - 18 h). After this time, the crucibles were withdrawn, cooled in

the desiccator during 60 min before being weighed. The operation has been renewed until obtaining a constant weight.

Measurement of degree brix (total soluble matter)

The degree Brix was measured using a refractometer (Euromex, Holland). A drop of liquid zoom-koom was deposited on the dial of the apparatus. The reading was done through the luminous indicator of the apparatus.

Mineral composition: P, Mg, Ca, Fe, Zn

Determination of phosphorus

(i) After mineralization of the organic matter with a mixture of perchloric acid ($HClO_4$) and oxygenated water (H_2O_2), the phosphorus of the sample was in the form of orthophosphate (H_3PO_4), which in acid medium combines with molybdate to give a phosphomolybdic complex $H_3P(MO_3O_{10})_4$. This complex in the presence of ascorbic acid was reduced to molybdenum blue, which allows a colorimetric determination (the intensity of the coloration depends on the phosphate content).
(ii) In a clean, and dry mattress, 1 g of sample, 4 mL of H_2O_2 and 2 mL of $HClO_4$ were introduced. The mineralization was carried out by gradual heating of 15-20 min at 50, 70, 90, 100 and 200°C on the heating plates until obtaining a yellowish or whitish solution with the release of whitish smoke. After cooling, the volume of mineralization was supplemented to 100 mL with distilled water.
(iii) In a tube test, 1 ml of the digestion product filtrate, 5 ml of distilled water, 4 ml of a solution composed of 10 ml of H_2SO_4 (6N), 10 ml of 2.5% ammonium molybdate and 10 ml of 10% ascorbic acid were introduced. The coloring was allowed to develop for 30 min and then the absorbance was read at 820 nm using the spectrophotometer. The phosphorus content was determined using a standard curve made from a stock solution of K_2HPO_4 at 20 mg / ml.

Determination of Magnesium, Calcium, Zinc and Iron contents

After wet mineralization, the different ions were assayed by atomic absorption. About 1 g of dried sample was digested in a 50 ml mixture with 4 ml of a mixed solution composed of perchloric acid ($HClO_4$) 60% and concentrated sulfuric acid (H_2SO_4) (7/1: V / V) and 15 ml concentrated nitric acid (HNO_3). Heating was gradual up to 345°C. After complete digestion, the mineralization was cooled; the volume was reduced to 50 ml after filtration. For the determination of calcium and magnesium, to 0.2 ml of filtrate, 4.8 ml of an aqueous solution of lanthanum (La_2O_3) 1% were added. The addition of lanthanum eliminated interferences of phosphorus and aluminum. The iron was diluted with distilled water. The atomic absorption spectrophotometer (Perkin-Elmer model 303) was used to read the absorbance at the following wavelengths: 422.7 nm for Ca, 285.2 nm for Mg, 248 nm for Fe and 213.9 for Zn. The contents were determined using standard curve for each element.

Microbiological analyzes of the samples

For the preparation of stock solutions, there were tenfold dilutions and inoculation in agar plates. For all the determinations, 10 g of the samples were homogenized in a stomacher with 90 ml of sterile peptoned buffered water. Tenfold serial dilution was prepared and spread-plated for microorganisms count. 1 ml of suitable diluted was use for spreading. Aerobic mesophilic bacteria (AMB) were enumerated in plates of Plate Count Agar (Liofilchem, Italy) incubated at 30°C for 72 h (ISO 4833, 2003).

Yeasts and Moulds were counted by cultivation on Yeast extract Glucose-Chloramphenicol Agar (Oxoid, England) after incubation at 25°C for 4-5 days according to ISO 7954 (1988) standard. Lactic acid bacteria (LAB) were counted by cultivation on De Man, Rogosa and Sharpe Agar (Liofilchem, Italy) incubated anaerobically in an anaerobic jar at 37°C, for 3 days according to ISO 15214 (1998) standard. Coliforms were enumerated on Violet Red Bile Agar (VRBA) (Liofilchem, Italy), incubated at 37°C (Total Coliforms) or 44°C (Thermotolerant coliforms) for 24 h. The total and thermotolerant coliforms were enumerated according to ISO 4832 (2006) and NF V08-060 (2009) respectively.

Sensory analysis of liquid zoom-koom and instant flour zoom-koom samples

Grading test on the formulation samples of liquid zoom-koom and instant flour zoom-koom

The purpose of this test was to classify the 04 formulations of liquid zoom-koom as well as instant flour zoom-koom according to the tasters preference. 40 tasters were asked to give their opinion on the samples. The formulation that received the highest rate was retained for controlled fermentation with *L. fermentum* starter culture.

Difference test on liquid zoom-koom and instant flour zoom-koom produced with *L. fermentum* strains SF9.5 AND SF6.2

The selected formulation retained during grading test was reproduced by using *L. fermentum* strains SF9.5 and SF6.2. This test aims to compare 02 liquid zoom-koom samples (one was produced with the *L. fermentum* strain as ferment and the second was prepared without *L. fermentum* and serves as a control); it aims also to compare 02 instant flour zoom-koom samples (one was produced with the *L. fermentum* strain as ferment and the second was prepared without *L. fermentum* and serves as a control). This difference test required a panel of 24 tasters as describes by Cochran and Cox (1957).

Profile test on liquid zoom-koom and instant flour zoom-koom produced using *L. fermentum* strains SF9.5 AND SF6.2

The aim of this test was to determine the preference of the panelists by comparing the acidity, the sweet taste and the spicy taste of liquid zoom-koom and instant flour of zoom-koom obtained with starter cultures. This test required 06 experienced panelists.

Statistical analysis

All data were subjected to ANOVA with XLSTAT-Pro statistical software 7.5.2 and the means were compared using the Student Newman-keuls test at the probability level $p<0.05$. The curves were obtained using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Biochemical, microbiological and sensorial characteristics of zoom-koom formulations

Acidity and pH

The pH of the liquid zoom-koom samples varied between 5.02 and 5.13 for a titratable acidity which varied between

1.21 and 1.89 g of lactic acid / 100 g of product (Table 6). The pH of instant flour zoom-koom varied between 5.11 and 5.70. The acidity varied between 0.81 and 1.21 g of lactic acid / 100 g of product (Table 7). These values showed that both types of zoom-koom are acidic beverages (pH<6). The acidification of the foodstuffs was a method used to stabilize the products. Many studies (Thompson and Weber, 1979; Champagne and Phillippy, 1989) reported the effect of acidification of food by improving the absorption of certain minerals.

Microbiological quality of liquid zoom-koom and instant flour zoom-koom formulations

The aerobic mesophilic bacteria counts of the 04 formulations of instant flour zoom-koom varied between 3.9×10^7cfu / g and 8.9×10^8 cfu / g, and the lactic acid bacteria counts between 1.8×10^7cfu / g and 3.0×10^8 cfu / g (Table 7). For total coliforms, their charge varied between 10 cfu / g and 4.7×10^4cfu / g. The thermotolerant coliforms ranged from 10 to 1.5×10^4 cfu / g. The yeasts and molds counts varied from 10 to 2.0×10^5cfu / g. No significant difference was observed ($p<0.05$). Based on aerobic mesophilic bacteria results, the 04 formulations of instant flour zoom-koom were unsatisfactory according to the recommendations by CECMA (2009) which limit is 10^6 cfu/g. Instant flour zoom-koom incorporated separately with milk and baobab pulp were conforms to the values recommended by CECMA (2009) which limited thermotolerant coliform to 10^2 cfu/g, but instant flour zoom-koom incorporated separately with tamarind. According to the standards on dried foods recommended by JORA (2009) who limited total coliform to 10^2 cfu/g, only instant flour zoom-koom incorporated with baobab pulp was conform. The yeasts and molds concentration in instant flour zoom-koom incorporated separately with baobab pulp, tamarind and traditional zoomkoom were found to be conforms to the recommendations by MSL (2018) (limit: 10^4 cfu/g).

For the 04 liquid zoom-koom formulations, the aerobic mesophilic bacteria varied between 1.5×10^7cfu / ml and 3.3×10^8cfu / ml (Table 6). The lactic acid bacteria varied between 9.0×10^6 and 1.7×10^8cfu / ml. The total coliforms ranged from 2.0×10^2 to 5.2×10^3 cfu / ml. Thermotolerant coliforms ranged from 10 cfu / ml to 2.3×10^3 cfu / ml. The yeasts and molds counts were 10 cfu / ml and 3.0×10^3 cfu/ ml. No significant difference was observed ($p<0.05$). The aerobic mesophilic bacteria as well as total coliforms concentrations in the 04 formulations (liquid zoom-koom incorporated separately with lemon, milk, baobab pulp and tamarind) were found to be non conforms to the recommendations by CECMA (2009) (limits: 10^6 cfu/ml for aerobic mesophilic bacteria and 10 cfu/ml for total coliforms). About thermotolerant coliform value, only liquid zoom-koom incorporated with lemon was conform to the recommendations by CECMA(2009) (limits: 10^2 cfu/mL). The yeasts and molds concentrations in the 04 formulations (liquid zoom-koom incorporated separately with lemon, milk, baobab pulp and tamarind) were found to be conforms to the recommendations by CECMA (2009) (limits: 10^4 cfu/ml).

GRADING TEST

The results of the ranking test of the 04 liquid zoom-koom and the 04 instant flour zoom-koom formulations were showed in Figures 3 and 4. Concerning the color of liquid zoom-koom, 51.7, 44.8, 37.9 and 36.7 % of the panelists liked zoomkoom incorporated separately with milk, baobab pulp, lemon and tamarind respectively. As for the flavor, 50, 41.4 and 40% of the panelists expressed a good appreciation on the liquid zoom-koom incorporated separately with baobab pulp, milk and tamarind respectively. The mouth feel (sensation of tasting) test showed a good appreciation of liquid zoom-koom incorporated with tamarind (44.8% of the panel), baobab pulp (42.9% of the panel), milk (41.4% of the panel) and lemon (36.7% of the panel). From these values, it appeared that the liquid zoom-koom with milk presented the best color; the liquid zoom-koom with baobab pulp presented the best aroma and the liquid zoom-koom with tamarind had the best mouth feel. 69, 44.2 and 34.9% of the panelists liked the color of instant flour zoom-koom incorporated separately with baobab pulp, tamarind and milk respectively whereas 35.7% of the panel enjoyed traditional instant flour zoom-koom. For the flavor or aroma of the instant flour zoom-koom incorporated with baobab pulp, tamarind and milk was well appreciated by 51.2, 31 and 38.1% respectively; 36.6% liked the aroma of the traditional instant flour zoom-koom. According to the sensation after tasting (mouth feel), the instant flour zoom-koom incorporated separately with baobab pulp and tamarind were found to be pleasant by 37.2% of the panelists, whereas the instant flour zoom-koom incorporated with milk and traditional instant flour zoom-koom were judged pleasant by 39.5 and 30.2% respectively. So, it appears that the instant flour zoom-koom incorporated with baobab pulp showed the best color and aroma and the instant flour zoom-koom incorporated with milk had the best month feel.

In sum, the liquid zoom-koom and instant flour zoom-koom incorporated with the baobab pulp were found to have the best organoleptic characteristics; so theses formulations were selected for controlled fermentation using *L. fermentum* strains.

Biochemical, microbiological and sensorial characteristics of zoom-koom PRODUCED BY controlled fermentation using *L. fermentum* strains (SF9.5 AND SF 6.2) As Starter Culture

Biochemical characteristics

pH and titratable acidity: The values of pH and

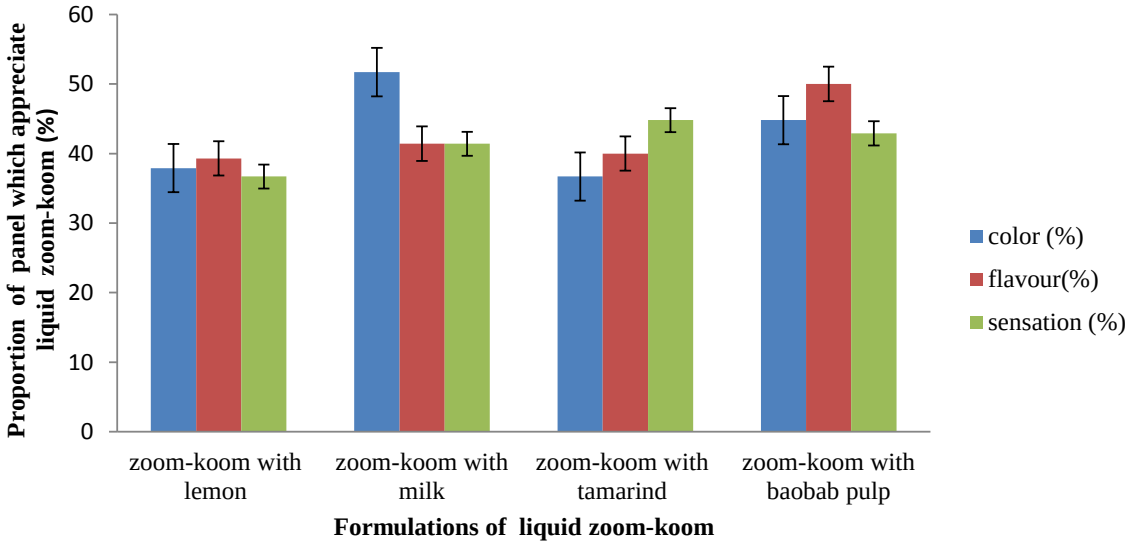

Figure 3. liquid zoom-koom samples ranking test.

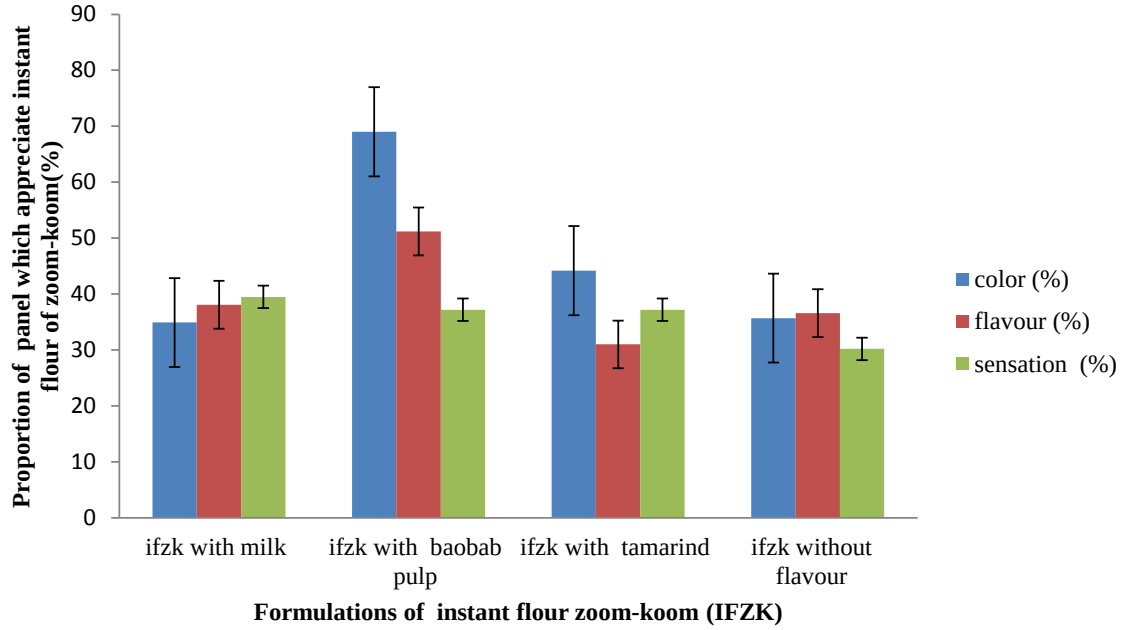

Figure 4. Grading test of instant flour zoom-koom.

titratable acidity of the zoom-koom samples ($p< 0.05$) obtained by controlled fermentation are presented on Table 3. The pH of the liquid zoom-koom samples (Table 3) varied from 3.69 to 5.39. The titratable acidity was between 0.71 and 1.51 g of lactic acid for 100 g of product. For instant flour zoom-koom samples, the titratable acidity varied from 0.42 to 0.49 g of lactic acid for 100 g of product. The pH value ranged from 3.07 to 3.9. These results showed that the traditional and controlled processing permitted to have an acid beverage. The lowering of the pH and the increase in acidity result from a lactic fermentation (Sawadogo-Lingani et al., 2010).

Proteins contents

The proteins contents of controlled fermented zoom-koom samples were respectively 25.83% dry matter (d.m) for the liquid zoom-koom without *L. fermentum*, 28.41%

for the liquid zoom-koom with *L. fermentum*, 4.57% for the instant flour zoom-koom without *L. fermentum* and 3.76% for the instant flour zoom-koom with *L. fermentum* (Table 4). These values showed that the liquid zoom-koom fermented by *L. fermentum* is richer in protein than the liquid zoom-koom without *L. fermentum.* On the other hand the instant flour zoom-koom without *L. fermentum* gave a content of protein higher than the instant flour zoom-koom fermented by *L. fermentum*.

Tapsoba and collaborators in 2017 obtained a protein content in the liquid zoom-koom fermented by *W. confusa/cibaria* and without *W. confusa/cibaria* ranging between 17.66 and 25.30% d.m respectively. The protein content of our samples was slightly higher than those reported by Tapsoba et al. (2017). The essential function of a food protein was to satisfy the needs for the nitrogen organization and essential amino acids. The quality of a protein was related primarily to its composition in essential amino acids.

Lipids contents

The fat contents of zoom-koom samples were 6.48% d.m (dry matter) and 5.04% d.m respectively for the liquid zoom-koom without *L. fermentum* and the liquid zoom-koom fermented by *L. fermentum* (Table 4). The contents were 2.62 and 4.62% d.m respectively for the instant flour zoom-koom without *L. fermentum* and the instant flour zoom-koom fermented by *L. fermentum*. These values showed that the fat content of the liquid zoom-koom without *L. fermentum* was higher than the fat content of the liquid zoom-koom fermented by *L. fermentum*. On the other hand the instant flour zoom-koom without *L. fermentum* contained less fat than the instant flour zoom-koom fermented by *L. fermentum*. Tapsoba et al. (2017), obtained fat contents of liquid zoom-koom without *W. cibaria/confusa* and with *W. cibaria/confusa* were 4.74 and 5.55% d.m respectively. The fat contents of our samples are similar to those obtained by Tapsoba and collaborators in 2017. A degradation of the lipids could cause the appearance of free fatty acids metabolized in aromatic compounds such as alcohols, ketones and aldehydes. The lipolysis leads to a development of the flavor. Several lactic acid bacteria isolated from the dairy products, mainly *Lactobacillus* spp presented a lipolytic activity (Montanari et al., 2013).

Total sugar and reducing sugars contents

The total sugar contents were 65.74% d.m (dry matter) and 64.83% d.m respectively for the liquid zoom-koom unfermented and the liquid zoom-koom fermented by *L. fermentum* (Table 4). Total sugar contents were 92.63 and 91.43% d.m respectively for the instant flour of zoom-koom unfermented and the instant flour zoom-koom fermented by *L. fermentum*. For the reducing sugars, the contents were 32.1 and 36.9% dm respectively for the liquid zoom-koom unfermented and the liquid zoom-koom fermented by *L. fermentum*. The contents were 29.88 and 47.43% dm respectively for instant flour zoom-koom unfermented and instant flour zoom-koom fermented by *L. fermentum*. In contrast, samples (liquid zoom-koom, instant flour zoom-koom) fermented using *L. fermentum* contained more reducing sugars than those unfermented. Tapsoba and collaborators in 2017 obtained total sugar contents in samples of liquid zoom-koom unfermented and liquid zoom-koom fermented by *L. fermentum* were 68.86 and 76.24% d.m respectively. The total sugar contents of our samples were lower than those obtained by Tapsoba et al. (2017). This difference ($p<0.05$) could be explained by the quantity of sugar added during the processing of zoom-koom, varying according to the processing. The first stage of food fermentation was the use of the glucids by the LAB. The LAB was able to degrade a broad range of oses like lactose and galactose, but also saccharose, maltose, glucose, fructose and α-galactosides. The major metabolite of this degradation was the lactic acid. Some few LAB like *L. fermentum, Lb. sanfranciscensis and Lb. pontis* were able to use the fructose as carbon source (Stolz et al., 1995).

Ash contents

The ash contents were 1.72 and 1.95% d.m respectively for the liquid zoom-koom fermented by *L. fermentum* and the unfermented liquid zoom-koom (Table 4). The values were lower for the unfermented instant flour zoom-koom (0.18% m.s) and the instant flour zoom-koom fermented by *L. fermentum* (0.19% d.m).The liquid zoom-koom contained more minerals than the instant flour of zoom-koom. Tapsoba and collaborators in 2017 found 0.32 and 0.69% d.m as ashes contents for unfermented liquid zoom-koom and liquid zoom-koom fermented by *L. fermentum* respectively. Our samples of liquid zoom-koom contained more ashes (1.72-1.95% d.m) than the samples analyzed by Tapsoba et al. (2017)(0.18-0.19% d.m). The difference observed could be explained by the effect of dilution and the manufacturing process.

Brix

The total soluble matter content (°Brix) of the samples of unfermented liquid zoom-koom and zoom-koom fermented by *L. fermentum* was 15 and 16.5% respectively (Table 4). Tapsoba et al. (2017) obtained 18% representing a mean value of total dried matter in unfermented liquid zoom-koom and fermented zoom-koom; this value was higher compared to the total soluble dried matter found in our samples of liquid zoom-koom.

Table 3. Evolution of the microbial load in the raw material and the finished product during the production process

Samples	Fermentation time	Aerobic mesophilic bacteria (cfu/g or cfu/ml)	Lactic acid bacteria (cfu/g or cfu/ml)	Total coliforms (cfu/g or cfu/ml)	Thermotolerant coliforms (cfu/g or cfu/ml)	Yeasts and molds (cfu/g or cfu/ml)	pH at 25°C	Titratable acidity (g of lactic acid /100 g)
Millet grains		$(8.2\pm4.1)10^{6}$	$(2.9\pm1.4)10^{3}$	$(9.7\pm4.8)10^{4}$	$(9.7\pm4,8)10^{4}$	$(1.0\pm0.5)10^{4a}$	5.62 ± 0.85^{a}	1.18 ± 0.15^{a}
Start of Soaking	0 h	$(3.6\pm1.8)10^{3a}$	$(3.5\pm1.7)10^{1a}$	$(6.0\pm3.0)10^{2}$	$(2.1\pm1.0)10^{1a}$	$(1.0\pm0.5)10^{1a}$	6.22 ± 0.007^{a}	0.54 ± 0.007^{a}
End of soaking	24 h	$(1.2\pm0.6)10^{9b}$	$(8.7\pm4.3)10^{7b}$	$(1.0\pm0.5)10^{1}$	$(1.0\pm0.5)10^{1a}$	$(7.7\pm3.8)10^{2a}$	4.29 ± 0.02^{b}	1.21 ± 0.00^{b}
Unfermented liquid zoom-koom	0 h	$(1.0\pm0.5)10^{6c}$	$(9.5\pm4.7)10^{5c}$	$(6.4\pm3.2)10^{2}$	$(3.0\pm1.5)10^{2b}$	$(1.4\pm0.7)10^{3b}$	5.39 ± 0.00^{c}	0.71 ± 0.00^{c}
	6 h	$(4.3\pm2.1)10^{6c}$	$(1.5\pm0.7)10^{6c}$	$(1.5\pm0.7)10^{2}$	$(9.0\pm4.5)10^{1b}$	$(2.5\pm1.2)10^{2b}$	$3.69\pm0.03^{c*}$	$1.29\pm0.08^{c*}$
Liquid zoom-koom using *L. fermentum* SF6.2 and SF9.5)	0 h	$(8.2\pm4.1)10^{7ab}$	$(8.4\pm4.2)10^{7ab}$	$(4.9\pm2.4)10^{4}$	$(4.9\pm2.4)10^{4ab}$	$(7.6\pm3.8)10^{5b}$	5.39 ± 0.00^{ab}	0.71 ± 0.00^{ab}
	6 h	$(1.0\pm0.5)10^{8ab}$	$(1.5\pm0.7)10^{8ab}$	$(5.8\pm2.8)10^{2}$	$(1.5\pm0.7)10^{2ab*}$	$(4.0\pm2.0)10^{1c*}$	$3.74\pm0.02^{ab*}$	$1.51\pm0.11^{ab*}$
Unfermented instant flour zoom-koom	0 h	$(4.3\pm2.1)10^{6ac}$	$(1.1\pm0.5)10^{6ac}$	$(4.3\pm2.1)10^{4}$	$(1.3\pm0.6)10^{4ac}$	$(5..1\pm2.5)10^{4ac}$	3.90 ± 0.00^{ac}	0.49 ± 0.00^{ac}
	6 h	$(5.0\pm2.5)10^{7ac}$	$(1.5\pm0.7)10^{8ac*}$	$(9.0\pm4.5)10^{3}$	$(3.3\pm1.6)10^{3ac}$	$(5.0\pm2.5)10^{3ac}$	3.83 ± 0.00^{ac}	$0.43\pm0.00^{ac*}$
Instant flour zoom-koom using *L. fermentum* SF6.2 and SF9.5	0 h	$(9.8\pm4.9)10^{5bc}$	$(9.0\pm4.5)10^{4bc}$	$(4.3\pm2.1)10^{4}$	$(1.6\pm0.8)10^{4bc}$	$(7.6\pm3.8)10^{5bc}$	3.90 ± 0.00^{bc}	0.47 ± 0.00^{bc}
	6 h	$(1.5\pm0.7)10^{6bc}$	$(7.7\pm3.8)10^{5bc}$	$(6.8\pm3.4)10^{3}$	$(2.7\pm1.3)10^{3bc}$	$(7.0\pm3.5)10^{3bc}$	$3.07\pm0.00^{bc*}$	0.42 ± 0.00^{bc}

Each column values having a common letter (a, b, c) are not significantly different according to the Student Newman Keuls test at the 5% threshold.
(*): Significant difference between values.

Minerals contents of zoom-koom samples

The minerals contents of liquid zoom-koom and instant flour zoom-koom fermented using *L. fermentum* on the one hand and unfermented liquid zoom-koom and instant flour zoom-koom on the other hand were showed in Table 5. The calcium contents varied between 0.218 and 0.435 g / 100 g d.m. The magnesium content varied between 0.039 and 0.065 g / 100 g d.m. The iron content was between 2.50 and 3.08 mg / 100 g. The phosphorus content was between 0.010 g and 0.012 g / 100 g d.m. From these results the liquid zoom-koom fermented using *L. fermentum* contained more calcium, magnesium and iron compared to the unfermented liquid zoom-koom. Similarly, instant flour zoom-koom fermented using *L. fermentum* contained more iron and phosphorus compared to unfermented instant flour zoom-koom. The micro-organisms played a role in the synthesis of vitamin factors, in the absorption of calcium, magnesium and iron. Several metals such as Fe^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+} and Zn^{2+} were necessary to the growth of the lactic bacteria (Salonen et al., 2014; Walsh et al., 2014).

Microbiological characteristics of controlled fermented zoom-koom

After 24 h of soaking the millet grains in water (Table 3), the concentration of aerobic mesophilic bacteria reached 1.2×10^{9}cfu / g. The soaking of the grains was always accompanied by a lactic fermentation, the drop in pH from 6.22 to 4.29 and the increase in titratable acidity (0.54 to 1.21 g of lactic acid / 100 ml); as well as the development of lactic acid bacteria whose concentration increased from 3.5×10^{1} cfu / g (beginning of soaking) to 8.7×10^{7} cfu / g at the end of soaking. During the same period, there was a drop of pH as well as a decrease of total coliforms from (6.0×10^{2} cfu / g to less than 10 cfu / g) and thermotolerant (2.1×10^{2} cfu / g to less than 10 cfu / g). This phenomenon was could be related to the acidification of the environment caused by the development of lactic acid bacteria.

Following the fermentation process for 06 h,

Table 4. Macronutrient composition of liquid zoom-koom incorporated with baobab pulp and instant flour zoom-koom incorporated with baobab pulp.

Samples	Proteins (%)/d.m	Lipids (%)/d.m	Reducing sugars (%)/ d.m	Total sugars (%)/ d.m	(%) Ashes /d.m	° Brix (total soluble dried matter)
Unfermented liquid zoom-koom	25.83±0.00^{a}	6.48±0.10^{a}	32.1±0.14^{a}	65.74 ±3.06^{a}	1.95 ±0.21^{a}	15.00 ±0.00^{a}
Liquid zoom-koom using inoculum (SF9.5 et SF6.2)	28.41±1.73^{b}	5.04±0.69^{a}	36.9±2.26^{b}	64.83 ±11.24^{a}	1.72 ±0.14^{a}	16.5 ±0.71^{a}
Unfermented instant flour zoom-koom	4.57±0.01^{c}	2.62±0.54^{b}	29.88±6.12^{c}	92.63 ±17.29^{b}	0.18 ±0.04^{b}	-
Instant flour zoom-koom using inoculum (SF9.5 et SF6.2)	3.76±0.02^{c}	4.62±0.54^{c}	47.43±13.77^{d}	91.43 ±4.70^{b}	0.19 ±0.01^{b}	-

(-): not determinate.
Each column values having a common letter (a, b, c) are not significantly different according to the Student Newman Keuls test at the 5% threshold.

Table 5. Minerals contents of liquid zoom-koom incorporated with baobab pulp and instant flour zoom-koom incorporated with baobab pulp.

Samples	Calcium (g/100 g d.m)	Magnesium (g/100g d.m)	Iron (mg/100 g d.m)	Phosphorus (g/100 g d.m)
Unfermented liquid zoom-koom	0.392±0.00^{a}	0.059±0.00^{a}	2.37±0.00^{a}	0.012±0.00^{a}
Liquid zoom-koom using *L. fermentum* SF9.5 and SF6.2	0.435±0.00^{a}	0.065±0.00^{a}	2.50±0.00^{a}	0.010±0.00^{a}
Unfermented instant flour zoom-koom	0.218±0.00^{b}	0.043±0.00^{b}	3.00±0.00^{b}	0.010±0.00^{a}
Instant flour zoom-koom using *L. fermentum* (SF9.5 et SF6.2)	0.218±0.00^{b}	0.039±0.00^{b}	3.08±0.00^{b}	0.011±0.00^{a}

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

microorganisms were counted at the beginning (0h) and the end (6 h) of fermentation. The aerobic mesophilic bacteria of the liquid zoom-koom samples varied from 1.0×10^{6} ufc / ml to 1.0×10^{8}cfu / ml (Table 3). The lactic acid bacteria varied from 9.5×10^{5} cfu/ml to 1.5×10^{8} ufc/ml. Total coliforms ranged from 1.5×10^{2} cfu/ml to 4.9×10^{4} cfu/ml. The thermotolerant coliforms varied from 9.0×10^{1} cfu/ml to 4.9×10^{4} cfu/ml. The yeasts and molds ranged from 4.0×10^{1} cfu / ml to 7.6×10^{4} cfu/ml.

The comparison of spontaneously fermented samples and the samples obtained by controlled fermentation using *L. fermentum* strains SF6.2 and SF9.5, in 06 h of fermentation revealed that natural fermentation reduced total coliforms by 76.56 % in 06 h, 70% for thermotolerant coliforms and by 82.14% for molds. In the same time, the controlled fermentation with *L. fermentum* SF6.2 and SF9.5 strains, reduced total coliforms by 98.82%, 99.69% for thermotolerant coliforms and by 99.69 % for molds. For instant flour zoom-koom, the microorganisms counts ranged from 9.8×10^{5} cfu/g and 5.0×10^{7} cfu/g (aerobic mesophilic bacteria), from 9.0×10^{4} cfu/g to 1.5×10^{8} cfu/g (lactic acid bacteria), from 6.8×10^{3} cfu/g to 4.3×10^{4} cfu/g (total coliforms), from 2.7×10^{3} cfu / g to 1.6×10^{4} cfu / g (thermotolerant coliforms), from 5.0×10^{3} cfu/g to 7.6×10^{5} cfu/g (yeasts and molds). We observed that natural fermentation reduced total coliforms by 79.07 and 74.61% for thermotolerant coliforms and by 90.2% for molds. The controlled fermentation of the instant flour zoom-koom using *L. fermentum* strains (SF6.2 and SF9.5) caused a reduction of 84.19% total coliforms, 83.12% thermotolerant coliforms and 46.15% molds. The effect of starter culture (*L. fermentum* SF6.2 and SF9.5) was less effective on the molds. The selected strains of *L. fermentum* used as starter cultures for zoom-koom production seem to express their antimicrobial capacity against coliforms, and yeasts and molds during fermentation.

The selected strains of *L. fermentum* SF6.2 and SF9.5 confirmed their acidifying capacity and their ability to inhibit the pathogens on the base of which they had been selected (Sawadogo-Lingani et al., 2008). The use of these strains as starter

Table 6. Microorganisms counts in the four formulations of liquid zoom-koom.

Samples	Aerobic mesophilic bacteria (cfu/ml)	Total coliforms (cfu/ml)	Thermotolerant coliforms (cfu/ml)	Lactic acid bacteria (cfu/ml)	Yeasts and molds (cfu/ml)	pH at 25°C	Titratable acidity (g of lactic acid/100 g)
Zoom-koom incorporated with lemon	$(1.5\pm0.7)10^{7a}$	$(5.2\pm2.6)10^{3b}$	$(1.0\pm0.5)10^{1a}$	$(9.0\pm4.5)10^{6a}$	$(3.0\pm1.5)10^{3a}$	5.03 ± 0.00^{a}	1.89 ± 0.00^{a}
Zoom-koom incorporated with milk	$(3.3\pm 1.5)10^{8b}$	$(4.4\pm2.2)10^{3b}$	$(2.3\pm1.1)10^{3b}$	$(1.7\pm0.8)10^{8b}$	$(1.0\pm0.5)10^{1b}$	5.05 ± 0.00^{a}	1.75 ± 0.00^{a}
Zoom-koom incorporated with tamarind	$(1.8\pm0.9)10^{8b}$	$(2.0\pm1.0)10^{2a}$	$(1.5\pm 0.7)10^{2ab}$	$(7.9\pm3.9)10^{7ab}$	$(1.5\pm0.7)10^{3a}$	$5.13\pm0,00^{a}$	1.21 ± 0.00^{b}
Zoom-koom incorporated with baobab pulp	$(1.2\pm0.6)10^{8b}$	$(3.5\pm1.7)10^{2a}$	$(1.5\pm0.7)10^{2ab}$	$(7.6\pm3.8)10^{7ab}$	$(2.5\pm1.2)10^{3a}$	$5.02\pm0,00^{a}$	1.48 ± 0.00^{b}

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

Table 7. Microorganisms counts, pH and titratable acidity of the four formulations of instant flour zoom-koom (IFZK)

Samples	Aerobic mesophilic bacteria (cfu/g)	Total coliforms (cfu/g)	Thermotolerant coliforms (cfu/g)	Lactic acid bacteria (cfu/g)	Yeasts and molds (cfu/g)	pH at 25°C	Titratable acidity (g of lactic acid /100 g)
IFZK incorporated with Milk	$(3.9\pm1.9)10^{7a}$	$(1.1\pm0.5)10^{3a}$	$(1.0\pm0.5)10^{1a}$	$(3.1\pm1.5)10^{7a}$	$(2.0\pm1.0)10^{5a}$	5.38 ± 0.00^{a}	1.08 ± 0.00^{a}
IFZK incorporated with baobab pulp	$(1.2\pm0.6)10^{8b}$	$(1.0\pm0.5)10^{1b}$	$(1.0\pm0.5)10^{1a}$	$(1.8\pm0.9)10^{7a}$	$(1.0\pm0,5)10^{1a}$	5.53 ± 0.00^{a}	0.94 ± 0.00^{a}
IFZK incorporated with tamarind	$(8.9\pm4.4)10^{8b}$	$(4.7\pm2.3)10^{4ab}$	$(1.5\pm0.7)10^{4b}$	$(3.0\pm1.5)10^{8b}$	$(2.5\pm1.2)10^{3b}$	5.70 ± 0.00^{a}	0.81 ± 0.00^{a}
IFZK (traditional formulation)	$(6.3\pm3.1)10^{8b}$	$(1.0\pm0.5)10^{4ab}$	$(3.7\pm1.8)10^{3b}$	$(3.0\pm1.5)10^{8b}$	$(1.0\pm0.5)10^{1a}$	5.11 ± 0.00^{a}	1.21 ± 0.00^{b}

Each column values having a common letter (a, b) are not significantly different according to the Student Newman Keuls test at the 5% threshol

cultures in the zoom-koom technology could improve the sanitary quality in order to assure consumers safety. Previous studies showed the presence of coliforms at high levels in the zoom-koom produced and sold in Ouagadougou city (Barro et al., 2002; Bsadjo-Tchamba et al, 2014) and similar beverages like kunun-zaki, a traditional millet-based drink from Nigeria (Gaffa, 2002; Elmahmood and Doughari 2007). In addition, zoom-koom processing in Burkina Faso does not include a pasteurization step to assure the safety of the products (Soma et al., 2017; Tapsoba et al., 2017). This study revealed that the 02 major ingredients that bring high levels of micro-organisms (pathogenic bacteria, lactic acid bacteria, yeasts and molds) were mint and ginger. The lactic fermentation could contribute to reduce pathogenic bacteria after 24 h of fermentation. Tapsoba et al. (2017) revealed that starters could considerably improve the hygienic quality of the zoom-koom.

The concentrations of aerobic mesophilic bacteria, total coliforms in unfermented liquid zoom-koom and instant flour zoom-koom on the one hand and in fermented liquid zoom-koom and fermented instant flour zoom-koom were found to be non conforms according the recommendations by CECMA (2009). However the concentrations of yeasts and molds in all the samples of zoomkoom were found to be conforms to the recommendations by CECMA (2009).

Sensorial characteristics of controlled fermented zoom-koom

Difference test: The results of the difference test were represented in Figures 5 and 6. From the difference test between the liquid zoom-koom obtained by natural or spontaneous fermentation used as control and the liquid zoom-koom fermented using *L. fermentum* strains (SF9. 5 and SF6.2), 11.11% of the tasters observed a slight difference between these two types of liquid zoom-koom and 44.44% of the panel observed a

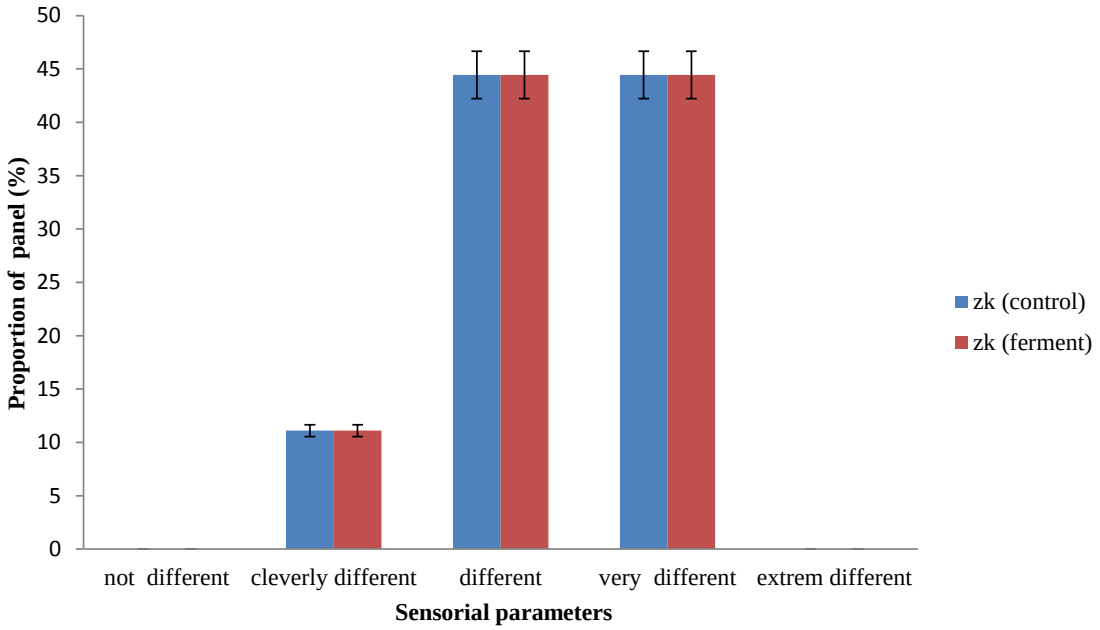

Figure 5. difference test from control concerning samples of liquid zoom-koom.
Test for difference from control between samples of unfermented liquid zoom-koom (control) and the fermeneted liquid zoom-koom (Z.K) using inoculum (S F 9.5 and SF6.2).

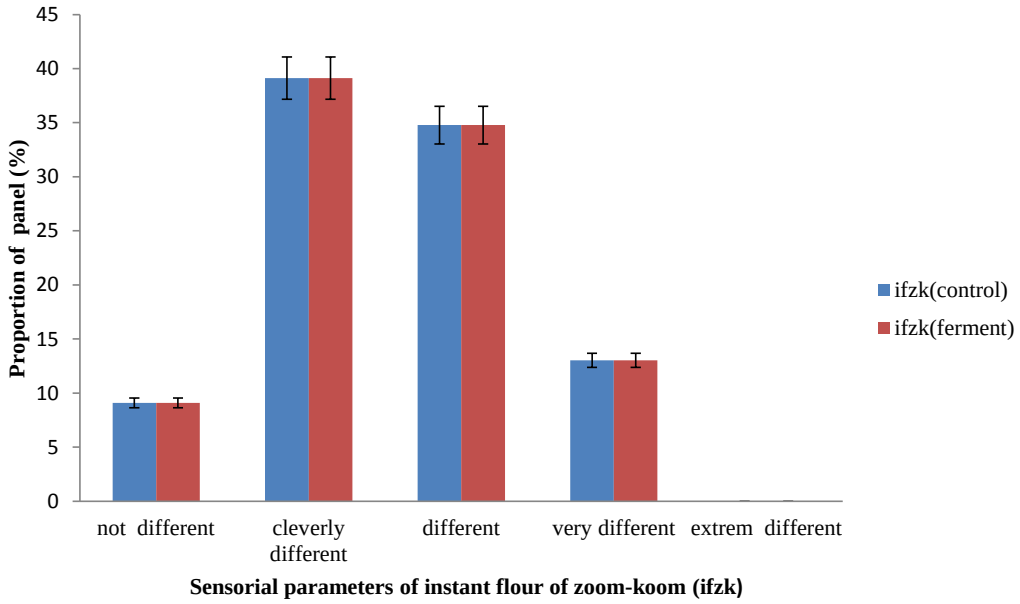

Figure 6. Difference test from control of instant flour zoom-koom.
Difference test between samples of unfermented instant flour zoom-koom (control) and instant flour zoom-koom fermented using *L. fermentum* SF9.5 and SF6.2

clear difference between these two types of liquid zoom-koom. From the difference- test between the instant flour zoom-koom (control) and fermented instant flour zoomkoom, 9.1% of the tasters did not notice any difference between these two types of zoom-koom. On the other hand, 39.13 and 34.78% of the panelists observed a slight and clear difference between these two types of instant flour zoom-koom; 13.04% of the panel observed a clear difference between these 02 types of instant flour zoom-koom. The results obtained in the

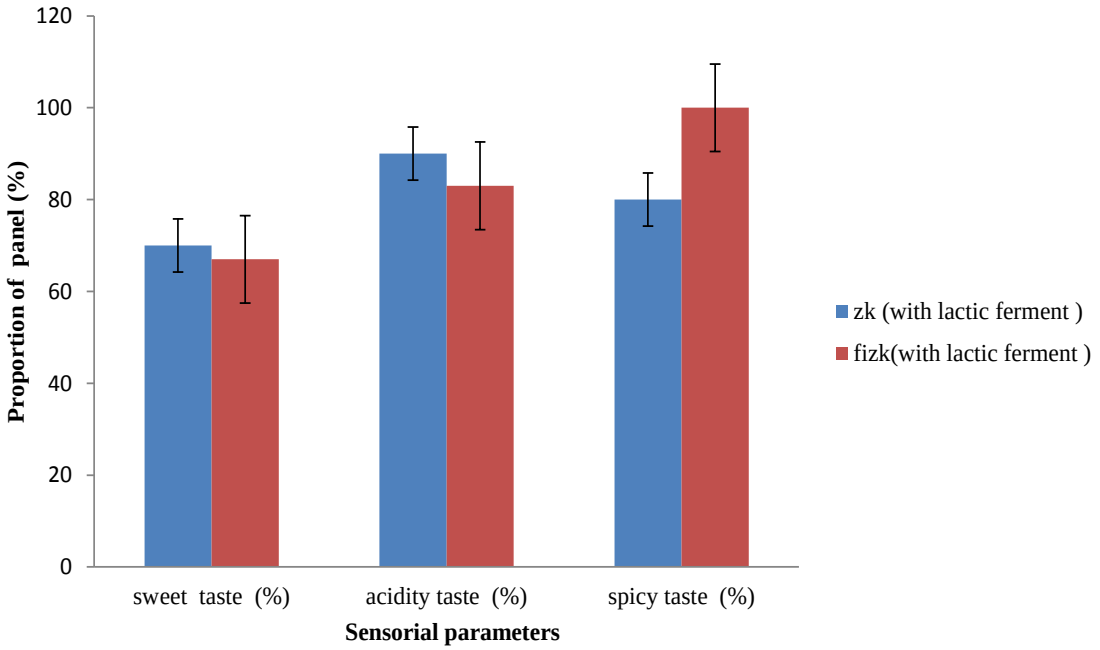

Figure 7. Profile test on liquid zoom-koom and instant flour zoom-koom produced using *L. fermentum* SF6.2 and SF9.5.
Profile test on liquid zoom-koom (Z.K) and instant flour zoom-koom (I.F.Z.K) produced using SF6.2 and SF9.5.

present study were comparable to those observed with kunun zaki in Nigeria by Gaffa and Ayo in 2002. Indeed, Gaffa and Ayo (2002) proved that the preparation of kunun zaki (beverage similar to zoom-koom) with starter cultures improved the organoleptic quality of the product.

Profile test: The results from profile test were shown in Figure 7. Concerning the liquid zoom-koom processed by controlled fermentation using *L. fermentum* (SF9.5 and SF6.2), 70% of the panelists appreciated its sweetened taste, 90% appreciated its acidulated taste and 80% appreciated its spicy taste. For instant flour zoom-koom processed by controlled fermentation using *L. fermentum* (SF9.5 and SF6.2), 67% of the panel appreciated its sweet taste, 83% its acidulated taste and 100% its spicy taste. These results showed that the liquid zoom-koom fermented with the selected *L. fermentum* (SF9.5 and SF6.2), had a sweet and acidulated taste more appreciated than the fermented instant flour zoom-koom. On the other hand the fermented instant zoom-koom flour would have a more pungent taste more appreciated than the fermented liquid zoom-koom.

Conclusion

The present study allowed comparing the hygienic and organoleptic quality of zoom-koom obtained by natural or spontaneous fermentation and that by controlled fermentation using *L. fermentum* SF6.2 and SF9.5 as starter culture. In terms of nutritional characteristics there was a slight increase in protein content, fat, reducing sugars, minerals such as calcium, magnesium and iron. In terms of hygienic quality, the study revealed that natural lactic fermentation prevented the development of microorganisms such as coliforms and molds. The controlled fermentation during 6 h did not eliminate all the pathogenic microorganisms in the final product zoom-koom but it confirmed the efficacy of the selection strains of *L. fermentum* SF6.2 and SF9.5. Furthermore, it was found that 6 h of controlled fermentation allowed to reduce considerably the bacteria such as coliforms. The present study showed also that the main sources of contamination in the zoom-koom were the raw material (millet grains) and the ingredients used (ginger, mint). The soaking of millet grains often overlooked by several processors for fear of having a very sour beverage plays a very important role in the elimination of the pathogens. It would eliminate more than 99% of the pathogenics and spoilage germs (coliforms and molds) present in the raw materials and ingredients in 24 h of soaking. On the organoleptic aspect, the present study contributed to develop 04 formulations of liquid zoom-koom and 04 formulations of instant flour of zoom-koom.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Stem bark of *Zanthoxylum zanthoxyloïdes* a possible substitute of root bark for the conservation of the species in Burkina Faso

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***Zanthoxylum zanthoxyloïdes* (Lam.) is used for some health purposes in Burkina Faso. However, human action threatened this species in its native environment and its regeneration is difficult by the way of population. The main focus of this study is to discriminate samples of *zanthoxyloïdes* from different plant populations in order to find good specimens for traditional medicine and thus contribute to the conservation of this species. Plant materials were collected from three study sites named Niangoloko, Orodara and Sidéradougou. Samples powder, mixed with potassium bromide was used for the Fourier-Transform Infrared Spectrometry (FTIR) analysis. Multivariate data analysis was performed to highlight differences in the spectral profile among plant organs. Then, vanillic acid characteristic signals in infrared were identified by using literature data. Results showed that leaf and stem bark spectra were significantly different ($p < 0.001$ and $p < 0.05$, respectively) among the study sites, while root bark spectra were almost identical ($p = 1.72$). Root bark and stem bark both indicated similar patterns under vanillic acid characteristic signals. The use of stem bark instead of roots can be a substitute for root to the sustainable management of this species in its native environment.**

Key words: *Zanthoxylum zanthoxyloïdes*, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, multivariate data analysis, vanillic acid.

INTRODUCTION

Zanthoxylum zanthoxyloïdes (Lam.) Zepern. and Timler (Rutaceae) is a West African plant species found in Burkina Faso, with limited geographic distribution, but mostly located in the western part of the country (Eyog Matig et al., 2006; Schmelzer and Gurib-Fakim, 2013).

In traditional medicine, this plant has shown its usefulness (Ynalvez et al., 2012) and well contributed to the health of the population. As an example, this plant is included in phytomedicines (FACA®, DREPANOSAT®) and is used for the health management of the sickle cell

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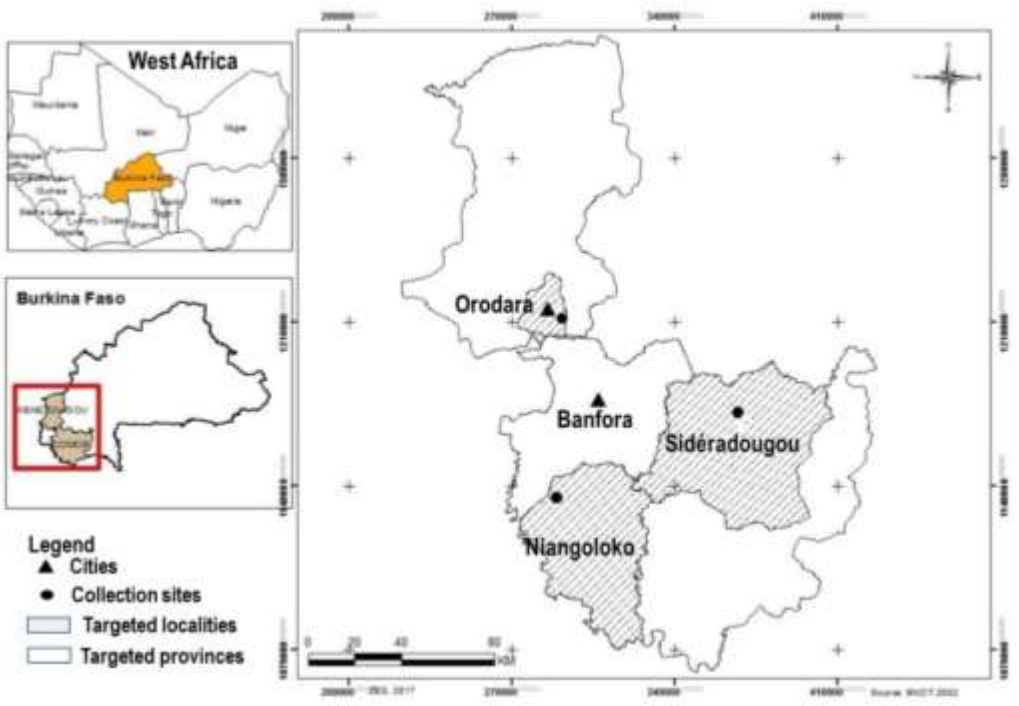

Figure 1. Samples sites.

disease (Imaga, 2010). Besides, ethnopharmacological investigations have been achieved on this plant and several active compounds such as vanillic acid and hydroxy-2-methyl-benzoic acid and derivatives were isolated from the leaf, stems bark and roots bark extracts (Nanfack et al., 2013; Ouattara et al., 2004; Guendéhou et al., 2018). These compounds play an important role in sickle cell disease treatment (Ouattara et al., 2009).

In another side, this plant is threatened and endangered in its native environment (Etsè et al., 2011; Thiombiano and Kampmann, 2010; Nikiema et al., 2010; Adomou et al., 2007*;* Compaoré et al., 2018). The root bark is the most valuable organs used by local populations, traditional healers and local pharmaceutical firms. This is the most important threat to the survival of this species in ecosystems (Diatta et al., 2014). The non-controlled harvesting, the massive destruction of plants by uprooting, and the lack of regeneration, reduced the population of *Z. zanthoxyloïdes* in its natural environment (Etsè et al., 2011).

Hence, it seems important to take initiatives for the sustainable use of this species. For this, spectroscopic techniques can be used to classify samples of any type due to its speed and simplicity, and its low-cost approach (Qi et al., 2017). For example, Fourier transform mid-infrared (FT-MIR) spectroscopy is an effective tool which can scan the chemical composition of a sample in the mid-infrared region (4000 to 400 cm^{-1}) and the fingerprint region created by this technique can give information on the sample's certainty quality (Grunert et al., 2016; Muhtar et al., 2016). In spectroscopy study, Principal Component Analysis (PCA) is the most commonly used technique for the discrimination of the samples (Kamil et al., 2015). PCA is used to show the distribution of the metabolites according to their wavenumbers. The samples with the similar scores are in the similar position while those of dissimilar scores are some distance away (Kamil et al., 2015).

This study aims to discriminate samples of *Z. zanthoxyloïdes* from three sites of Burkina Faso for the selection of better specimen for conservation (regeneration, propagation, cultivation, etc).

For this purpose, this study consisted of a clustering plant samples of *Z. zanthoxyloïdes* collected from three sites of Burkina Faso, and (ii) a determination of the spectral part that discriminates clusters. (iii) In addition, literature data of vanillic acid signals were estimated in the plant parts, to know the distribution of these useful compounds.

MATERIALS AND METHODS

Sites and sampling

Samples were collected in western regions, Niangoloko (N), Sidéradougou (S) and Orodora (O). One hundred and eight (108) samples were collected from twelve (12) randomly selected mature trees per site in fours defined plot (20 m × 250 m). The study sites are as shown in Figure 1. The annual precipitation of the three sites varied from 1000 to 1176 mm. The type of soil was fine particles, eroded and clay soil in Niangoloko, Siéderadougou and

Table 1. Vanillic acid wavenumber and intensity in infrared study.

Active principle	Wavenumber (Intensity)
Vanillic acid	3486 (33); 3099 (58); 2956 (10); 2924 (4); 2854 (11); 2849 (82); 1683 (17); 1598 (19); 1524 (20); 1465 (37); 1466 (33); 1435 (15); 1378 (37); 1299 (16) 1293 (19); 1282 (18); 1239 (20); 1206 (20); 1113 (46); 1030 (35); 919 (62); 883 (62); 820 (68); 807 (65); 768 (49); 766 (46); 722 (74); 689 (72); 637 (66); 611 (66); 583 (77); 542 (70)

Orodara, respectively.

Stem bark samples were taken off with a cutter (30 cm length, 7 mm width) from the median part of the trunk. Leaf and root bark have been collected manually and by using a scissor and a hoe, respectively. All samples were achieved by applying good agricultural and collection practice (WHO, 2004).

Sample preparation

Specimen of tree samples from Niangoloko, Orodara and Sidéradougou were collected and deposited, respectively under the voucher references 3061, 3062 and 3063, in the herbarium of Natural History Laboratory of National Center for Scientific and Technology Research (CNRST). Then, all samples were dried for 2 weeks in a drying oven at 30 to 35°C. The samples were powdered using a mixer (blender SQBL-100, China) to obtain fine particles with homogenate size (≤ 20 mm) according to a method previously described (Rana et al., 2008). Powder samples were stored in a desiccator over dry silica gel until it was used (n=3×36 independent samples).

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectral recording and multivariate data analysis

ATR-FTIR spectra were obtained using NICOLET™ iS™ 5 spectrometer (USA) in the range from 600 to 4000 cm^{-1}. 10 mg of each sample was used to make pellets (10 mm) for spectral recording. Each spectrum was obtained in triplicates (n=3). SIMCA P+ v (12, Sweden) software was used for PCA according to previous methods (Nacoulma et al., 2013; Rana et al., 2008).

The region (1800 to 600 cm^{-1}) and the characteristic signals of one active principle vanillic acid (AIST, 1999) shown in Table 1 were used to cluster samples in a PCA.

RESULTS AND DISCUSSION

Spectra visual observation

The main characteristics of spectra are as shown in Figure 2. The leaf spectra profile from Orodara was different to Niangoloko and Sidéradougou in the range between 3800 and 3400 cm^{-1}, 2400 and 2200 cm^{-1} and 1600 and 1500 cm^{-1}. The stem bark spectra profile from Orodara were very close to the one observed in Niangoloko except in the spectral region between 3800 and 3400 cm^{-1}, while in stems bark samples from Sidéradougou, spectra were different in the range between 3800 and 3400 cm^{-1}, 2400 and 2200 cm^{-1} and 1600 and 600 cm^{-1}.

The spectra profile from roots for the three sites was almost similar except in the region 3800 to 3400 cm^{-1}.

As observed*, Z. zanthoxyloïdes* organs spectra profiles exhibit three characteristics zones: 3800-3400 cm^{-1}, 2300 cm^{-1} and 1600-600 cm^{-1}. The region 3800-3400 cm^{-1} corresponds to OH bending band (Cebi et al., 2019). The region 2300 cm^{-1} indicates alkyne and nitrile region (Suzuki et al., 2005). According to previous studies, the region around 1650 and 1100 cm^{-1} corresponding to a fingerprint region indicates the major cell wall components, such as cellulose, a polyosidic compound, and lignin, a polyphenolic compound (Rana et al., 2008).

According to the spectra pattern, there was a difference within the leaf and stem bark between 1800 and 600 cm^{-1} but root bark spectra were similar within the various provenances. A possible explanation for this is that the distribution of the metabolites in the plants parts is affected by climate and soil (Sampaio et al., 2016).

PCA in the region 1800-600 cm^{-1}

For further information about the variability among samples, PCA was performed on the corresponding first derivative FTIR spectra in the 1800 to 600 cm^{-1} region (Figure 3). Of particular interest was the fingerprint region which includes the absorbed O-H vibration, C-H deformation in lignin and carbohydrates, C-H deformation in cellulose and hemicellulose, C-O stretch in lignin, C-O-C vibration in cellulose and hemicellulose and C-O stretch in cellulose and hemicellulose (Gupta et al., 2015).

The score plot of the first two principal components explained 62.24% of the variability in the dataset (PC1: 39.45% and PC2: 22.79%) for leaves (Figure 3a). Concerning stem bark, PC1: 40.88% and PC2: 30.38% contributed to 71.76% of the spectra variability (Figure 3b). For root bark, the first two principal components (PCs), together accounted for 78.42% of the total variability present in the spectra (Figure 3c).

However, almost all Orodora root bark samples were located on the positive side of PC1. Along the PC2 axis, we observed that all the stem bark samples from Orodara were on the negative side of PC2, while the signals from stem barks from Niangoloko and Sidéradougou are found on both sides of PC2. All the root samples from

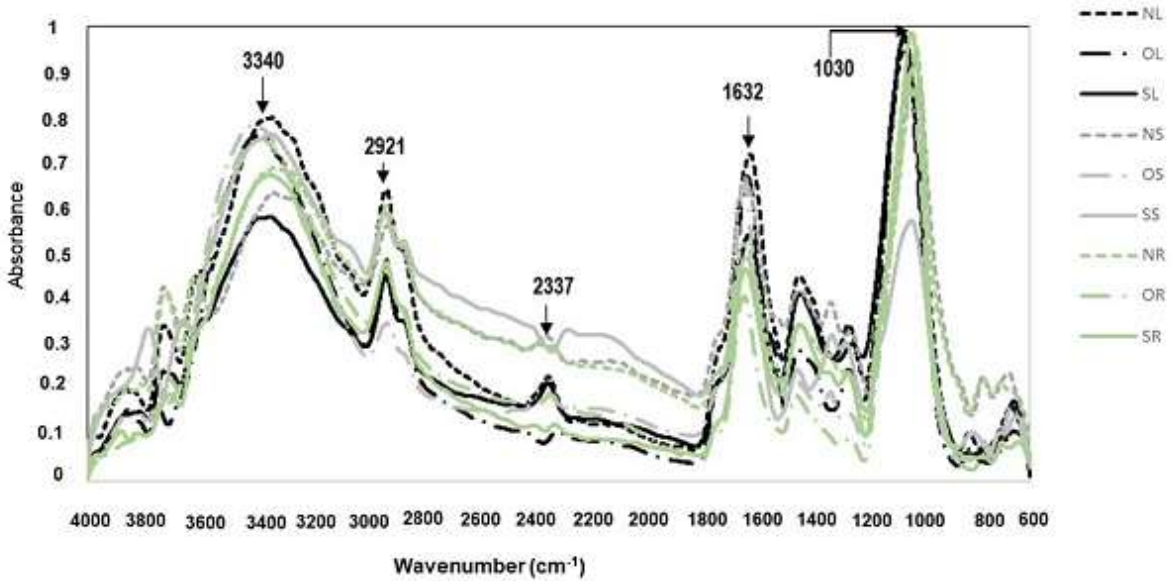

Figure 2. FTIR spectra of *Z. zanthoxyloïdes* leaf, stem bark and root bark in the range from 4000 to 600 cm^{-1} and fingerprint region, respectively. Each spectrum is a mean of spectra from 12 individual trees sampled at Niangoloko (N), Sidéradougou (S), Orodara (O). L corresponds to Leaf, S for Stem bark and R for root bark.

Niangoloko were located on the positive PC2 axis. The root samples from Sidéradougou and Orodara were found to be on the negative side of PC2.

Regarding PC1 and PC2 axes, stem bark from Orodara was distinct to stem bark from Niangoloko and Sidéradougou. These results indicate that there are two groups of metabolites in stem bark. The samples from Orodara were in most cases narrowly grouped together in contrast to those derived from Niangoloko and Sidéradougou. One peculiarity of the Orodora site was the presence of dense clay soil whereas at both other sites soils with loosely packed particles dominated.

The PCA scores based on infrared signals in the range of 1800 to 600 cm^{-1} were tested by a one-way-analysis of variance (ANOVA) to assess spectra variability (Table 2). A difference between leaf spectra and locations could be detected with high reliability ($p = 2.59\times10^{-6} < 0.001$) and as well as a difference within stem bark from *Z. zanthoxyloïdes* metabolites' part profiles across collection sites ($p = 0.01 < 0.05$). No difference for root bark was observed ($p = 1.72$). These results confirm our visual observation of the difference among leaf and stem bark in the range between 1800 and 600 cm^{-1} and resemblance among root barks across the various sites.

This variability of the spectra within the same plant organ can be further interpreted by inspecting the loadings corresponding to PC1 (Figure 4 and Table 3) to identify the signal. An examination of the loadings plot revealed that the variance along the first principal component was mainly driven by signals in the regions 1500 and 1233 cm^{-1} for leaf samples. These regions are characterized by aromatic skeletal vibration of C=O stretch at 1500 cm^{-1} and stretching C-H vibrations around 1233 cm^{-1} of lignin, respectively (Rana et al., 2008; Carballo-Meilán et al., 2014; Shi et al., 2012; Leopold et al., 2011).

The principal wavenumbers found in the analysis of stem bark were 750, 1030, and 1596 cm^{-1}. Absorption bands in these regions are usually due to C-H deformation in cellulose and hemicellulose (Pandey and Pitman, 2003). The peaks from root bark were in the bands around 1030, 1125 and the 1650 cm^{-1}. Peaks in those regions are characteristic of lignin C-H and aromatic skeletal vibration combined with C-H in-plane deforming and stretching (Rana et al., 2010; Shi et al., 2012; Lammers, 2008; Carballo-meilan et al., 2014). These results indicate all cell wall constituents from leaf, stem bark and root bark in the region 1800 to 600 cm^{-1}.

Targeted analysis

FTIR spectroscopy multivariate data analysis was used to discriminate *Z. zanthoxyloïdes* plant parts under vanillic acid characteristic signals found in the infrared study. As shown in Figure 5a, the two first PCs explained 71% of the spectra dataset variance (PC1: 51.56%; PC2: 20.30%) and PC2 explained mainly the discrimination between clusters. Indeed, most of the stem bark and root bark samples clustered closely together regardless of the sampling site on the positive side of the PC2 axis. In contrast, the leaves samples from the various collection sites built up a close group on the negative side of the PC2 axis. The loading plots under PC2 were analyzed to

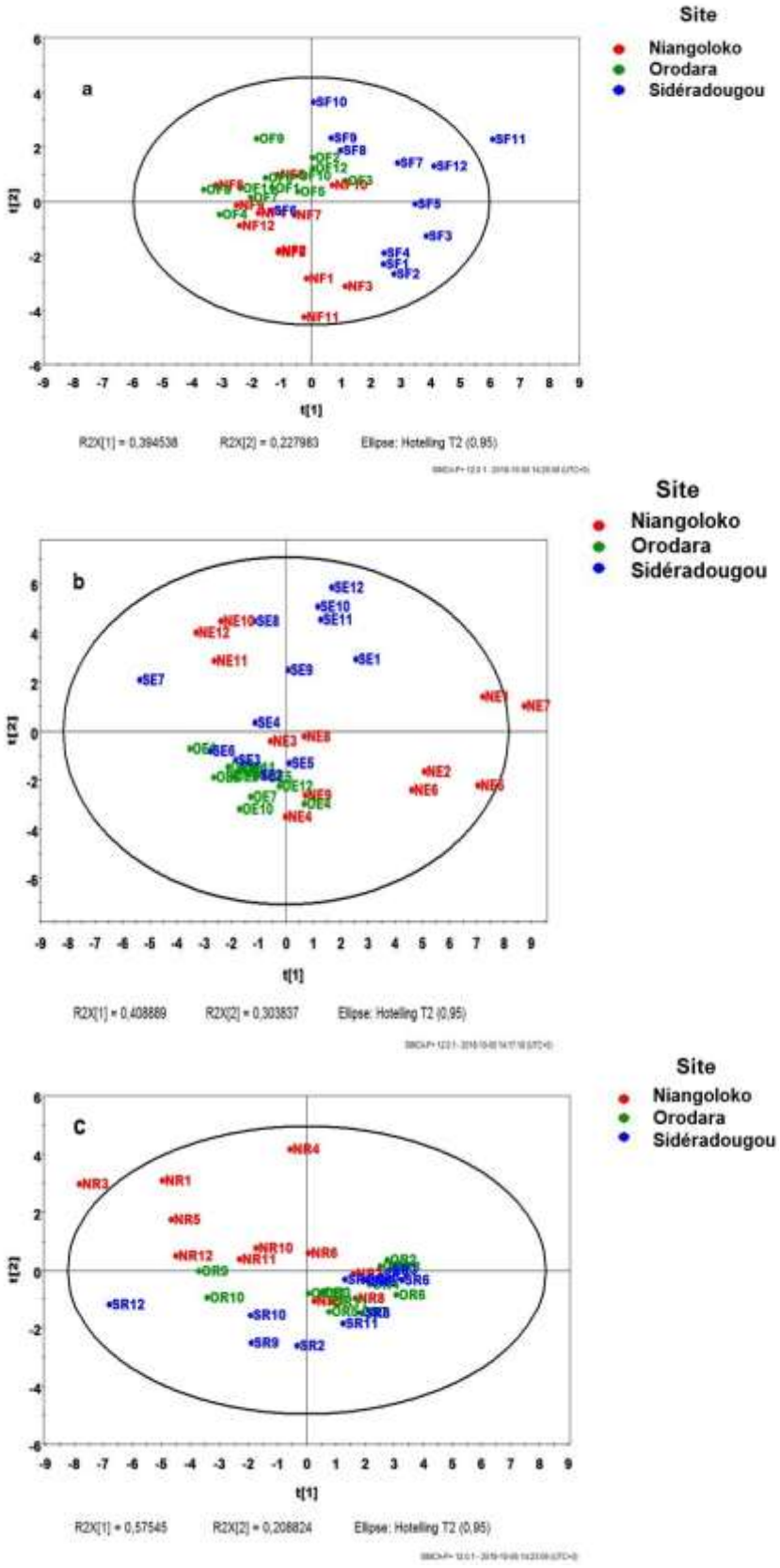

Figure 3. PCA analysis of *Z zanthoxyloïdes* leaf (a), stem bark (b) and root (c) in the fingerprint region (1800-600 cm^{-1}). O for Orodara, S for Sidéradougou, N for Niangoloko. Plant parts: L=Leaf, S= stem bark, R= Root. The colors refer to sites

Table 2. The one-way ANOVA analysis of PCA scores based infrared signals in the range 1800-600 cm^{-1}

Organs	DF	F	*p*-value	Signification
Leaf	35	19.48	<0.001	HS
Stem bark	35	5.26	0.01	S
Root bark	35	1.72	0.19	NS

DF: Degree of freedom, F: Fisher coefficient, HS: highly significant, S: significant, NS: non-significant.

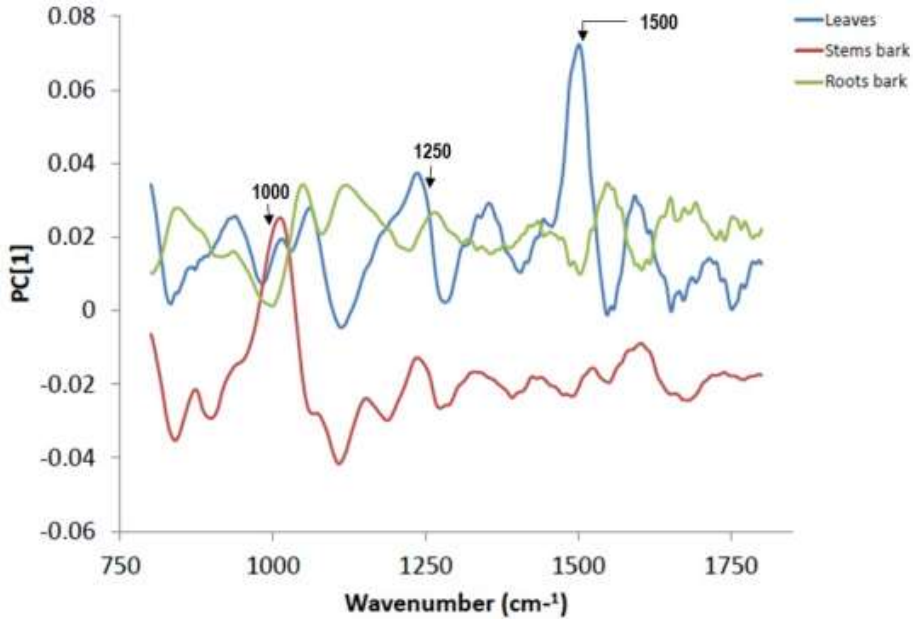

Figure 4. The loadings profiles of the first principal component (PC1) for leaf, stem barks and roots in the region (1800-600 cm^{-1}). The colors refer to plant parts.

highlight the characteristic signals. Examination of the PC2 loadings plot (Figure 5b) showed that the variance along this component is mainly driven by signals around 1030 and 1100 cm^{-1}. The bands in the region 1030 cm^{-1} region are due to aromatic C-H in plane deformation, guaiacyl-type, C-O deformation, and primary alcohols (Rana et al., 2008), while those in the region 1100 cm^{-1} can be assigned to C–O and C–C stretching modes (Nik Norulaini et al., 2011; Leopold et al., 2011).

With the particularity of spectra in the region around 1030 cm^{-1} (Rana et al., 2008), the relative average content of vanillic acid in plant parts (Figure 6) was evaluated according to signal strength in this region. The results showed that the relative amount of vanillic acid in stems and roots bark was relatively similar within an absorbance range of 0.4 to 0.95 with plus two standard deviations (+2SD) above the average, while leaves contained less vanillic acid within an absorbance range of 0.1 to 0.4 with two standard deviations (-2SD) below the average (Figure 6).

The present results can confirm the widespread use of roots bark for phytomedicine purposes containing vanillic acid related to sickle cell disease. Several studies have already shown that the active principle vanillic acid from *Z. zanthoxyloïdes* was mainly found in the roots bark (Ejele et al., 2012; Ameh et al., 2012; Adegbolagun and Olukemi, 2010; Ouattara et al., 2009; Elekwa et al., 2005). Then, referring to the present work, stem bark can be used as an alternative to root bark and avoid the uprooting of the species that will contribute to its sustainable management in its environment.

Conclusion

Using FTIR based metabolomics on *Z. zanthoxyloïdes* samples (leaf, stem bark and root bark) from three different sites in the south-western region of Burkina Faso, differences among plant specimens were highlighted. Leaf spectra were highly different (*p* =

Table 3. Band assignments in the mid-infrared region of *Z. zanthoxyloïdes* (leaf, stem bark and root bark)[1].

Wavenumber (cm^{-1})	Band origin	Organs
1748	Hydroxyl groups	Root bark
1730	C=O stretch in unconjugated ketones, carbonyls and in ester groups	Leaf
1696	Aromatic skeletal vibration plus C=O stretch, Aldehydes, Ketones, Carboxylic acids, Esters	Root bark
1650	Aldehyde (CHO) (lignin)	Root bark
1596	Aromatic skeletal vibration plus C=O stretch	Stembark
1596	Aromatic skeletal vibration plus C=O stretch	Leaf
1550	NO2 , Nitro compounds	Root bark
1519	Aromatic skeletal vibrations	Stem bark
1500	Aromatic skeletal vibration plus C=O stretch	Leaf
1429	Aromatic skeletal combined with C-H in-plane deforming and stretching	Root bark
1425	Aromatic skeletal vibrations combined with C-H in plane deformation	Stem bark
1350	C-O stretching vibration (cellulose and hemicellulose)	Leaf
1330	S ring plus G ring condensed	Stem bark
1256 - 1376	The C-H vibrations of lignin	Root bark
1233	C-O stretching, The C-H vibrations of lignin,	Stem bark and leaf
1184	Typical for HGS lignins; C=O in ester groups	Stem bark
1125	aromatic skeletal and C-O stretch	Root bark
1100	C-O-C, C-O dominated by ring vibration of carbohydrates	Stem bark
1060	C-O stretching vibration (cellulose and hemicellulose)	Leaf
1030	Aromatic C-H in plane deformation, guaiacyl type and C-O deformation, primary alcohol, C-O-C, C-O dominated by ring vibration of carbohydrates	Stem bark and root bark
930	No information available	Root bark
926	No information available	Leaf
875	No information available	Stem bark
840	Aromatic C-H out-of-plane deformations of the 1, 3,4,5-substituted rings associated with the syringyl nuclei	Root bark
800	No information available	Leaf
750	Cutin	Stem bark

[1]Band assignment based on Carballo Meilán et al. (2014), Rana et al. (2008, 2010), Lammers (2008), and Pandey and Pitman (2003). FTIR : Fourier transform infrared.

$2.59\times10^{-6} < 0.001$) among the three sites, as were stem bark spectra ($p = 0.01 < 0.05$) among the collection sites while root bark spectra were almost identical ($p = 1.72$) in the three provenances.

The discrimination of samples under vanillic acid characteristic signals, metabolite known to be important for the sickle cell disease treatment, on the other hand, was not correlated to geographic sites, but to plant organs. Root bark and stem bark under vanillic acid characteristic signals generated similar peaks around wavenumber 1030 cm^{-1} within an absorbance range of +2SD from average. Thus, stem bark could serve as a substitute for root as a resource for generating active ingredients from *Z. zanthoxyloïdes,* for the treatment of sickle cell disease. This would avoid detrimental uprooting of *Z. zanthoxyloïdes* plant populations and thus decisively improve the sustainable management of this species in its native environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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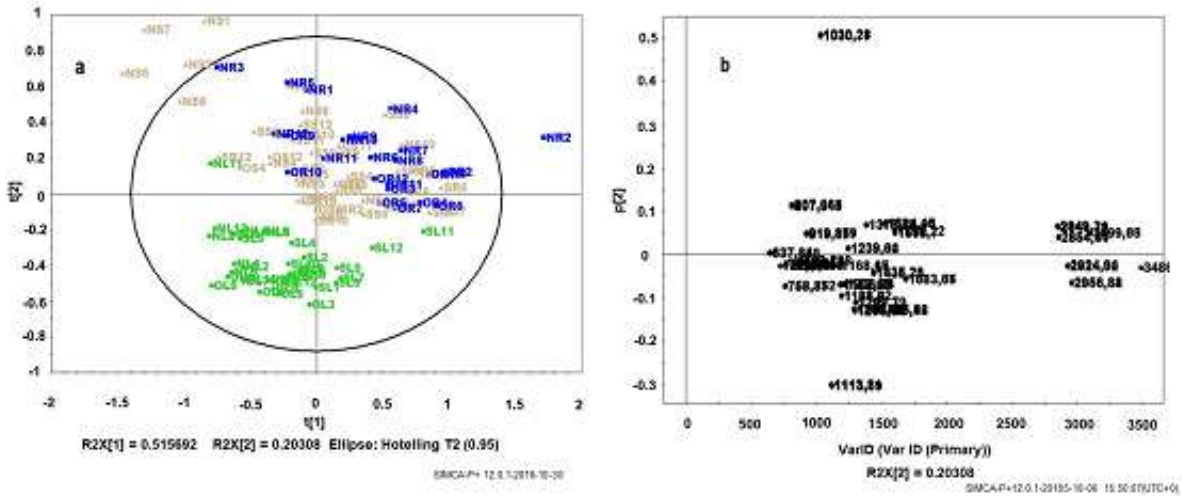

Figure 5. PCA score plot of *Z.zanthoxyloïdes* plant parts (leaf, stem bark and root) under acid vanillic signals (a), Loading plot of spectral variables corresponding to PC2 (b). O for Orodara, S for Sidéradougou, N for Niangoloko. Plant parts: L=Leaf, S= stem bark, R= Root bark. The colors refer to the plant parts.

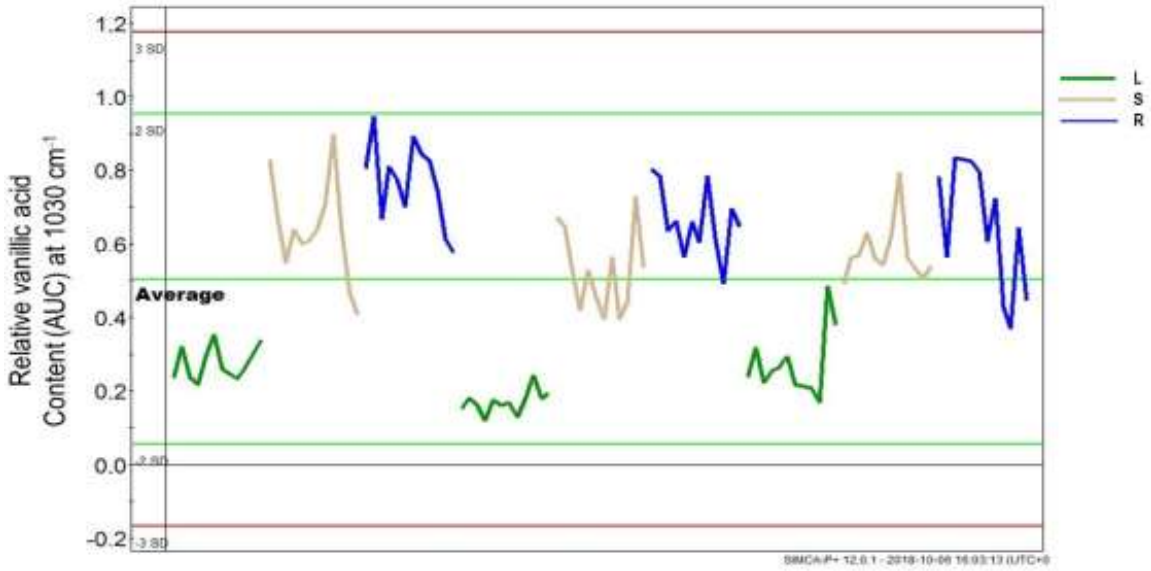

Figure 6. Relative vanillic acid content in plant parts at 1030 cm^{-1}. AUC: Area under curve. The color refers to plant parts. SD: standard deviation. L=Leaf, S= stem bark, R= Root bark.

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