

Effect of seeds soaking on the acidification of resulted flour (*Zea mays*) by wild fermentation and determination of acidification capacities of isolated lactic acid bacteria

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Abstract. This work is related to the effect of soaking of corn seeds in water on the acidification of resulted flour and on the acidifying power of fermentative microbial strains. The study was focused on two types of flour. The first flour was made from previously two days soaked corn kernels in water and the second flour was resulted from untreated corn seeds. These two types of flour were subsequently submitted to spontaneous fermentation during three days and changes in titratable acidity, pH and lactic flora in both flours were measured. At the start of fermentation process, evaluation of acidity characteristics showed that the flour resulted from soaked corn seeds (SMF) was more acidic than that obtained from untreated corn seeds (NSMF). Acidity of the two flours increased from the start until to the end of fermentation process. Furthermore, changes in the lactic acid bacteria population of flour SMF were not significant. But, the growth of lactic bacteria in flour NSMF was strongly significant. Consequently, flour SMF may have probably better stability than flour NSMF during storage. During the spontaneous fermentation process of the two flours, predominant microbial strains were cocci. But lactic acid bacteria might be mainly responsible of acidification of fermentative flour. The acidifying power of thirty strains was tested in a broth made from soaked corn seeds. Classification of lactic acid bacteria according to their acidifying power allowed defining four groups. Changes in the acidity of corn flour fermented by each defined group were followed for 24 h of fermentation. The acidity varied respectively from 0 to 0.45% for group 1, 0 to 0.42% for group 2, 0 to 0.40% for group 3 and 0 to 0.39% for group 4. The group of lactic acid bacteria with the best profile of acidification was group 1.

Keywords: Bacteria, soaking, fermentation, corn, flour.

INTRODUCTION

Cereals occupy a prominent place in the food security in the world, particularly in Africa. The main consumed

cereals are rice, wheat and maize (Bricas et al., 1995). If rice and the corn result mainly from the importation, the

corn is rather locally cultivated in various African geographical regions. The success of the corn is firstly due to its ease of culture and its productivity notably higher than those of wheat and coarse grains (millet and sorghum) it replaced (Assiedu, 1989). The corn plant is especially cultivated for its grains which can be consumed directly or transformed into flour. The corn flour is often used for making porridge for adults feed, but for children also. On the sidelines of corn porridge, it is also used in the manufacture of "tôh" which is the staple food in northern regions of Côte d'Ivoire. In Latin America such as in the Iberian Peninsula, the corn flour is used to make bread and cake (Bressani, 1972). All these applications show the importance of corn flour in human alimentation. But the post harvest treatments of this crop (hygienic and sanitary conditions) and storage under humid atmosphere of the flour in the sub-Saharan Africa region promote the growth of microorganisms that could cause diarrhea disease affecting infants (Caplice and Fitzgerald, 1999). Moreover porridges made from mainly corn flour are considered to be insipid by consumers who have resorts to the addition of acidic ingredients such as lemon to enhance their taste (Bressani, 1972). In order to improve the organoleptic qualities and to extend the storage duration of this meal, acidification processes were often applied (Hisiao and Siebert, 1999). There are two main methods of acidification of food: the acidification by the addition of organic acids and acidification by fermentation. The main problem resulting from the use of organic acids is the high concentration required to inhibit pathogenic bacteria or undesirable microorganisms. And then this chemical acidification is sometimes inconvenient for consumers (Kobilinsky et al., 2007). On the other hand, acidification by fermentation, with its lower cost than chemical acidification, allows the rapid proliferation of lactic acid bacteria producing organic acids and antibacterial substances (Hisiao and Siebert, 1999). Moreover, studies by Mensah et al. (1990) have shown that the use of starter culture for fermentation of infantile flours reduces the risks of malnutrition and diarrhea which are the main causes of the infant mortality in developing countries.

If the fermentation appears as an efficient method to improve sanitary quality, safety and taste of foodstuffs, the raw material must be of a good microbiological quality and that the microorganisms involved might be potentially capable to inhibit the growth of pathogenic micro-organisms by acidification. It therefore appears necessary to improve the process of acidification during the fermentation.

The main objective of our study is to evaluate the effect of soaking of the seeds on the acidification of corn flour by fermentation and the acidifying power of lactic acid bacteria isolated from resulted corn flour to improve the acidification process of flour during the fermentation. Specifically, the ideas are related to:

1. the improvement and the control of the acidification process of corn flour during the fermentation

2. the following and measurement of the changes in total acidity of fermenting corn flour by the evaluation of pH and titratable acidity

3. the isolation and numeration of lactic acid bacteria population in different corn flours.

4. the evaluation of the acidifying power of lactic acid bacteria isolated from fermented corn flours.

MATERIALS AND METHODS

The material used for this work is the white maize (*Zea mays*) purchased at the market of Treichville a district located at south of Abidjan (Côte d'Ivoire) during 2012. The culture medium used was MRS agar (Man Rogosa Sharp) for the detection of lactic acid bacteria. The dilutions were performed using Buffered Peptone Water (BPW).

Description of the preparation process and fermentation of flour

Two kilograms (2 kg) of corn grains are divided into two batches of one kilogram. After dehusking, the grains of the two batches are sorted to withdraw the undesirable elements. One of the batches of grains is soaked in two liters of water during two days while the other does not undergo any technological treatment. The two batches of grains were dried using oven drying and then ground to give NSMF (Not Soaked Maize flour) and SMF (Soaked Maize Flour) respectively. Then, 500 g of each flours obtained are mixed with two liters of water and placed in a closed container. The two types of flours are left to ferment during three days. Physico-chemical changes (pH and titratable acidity) and microbiological tests (counts, isolation, morphological and biochemical characterization of lactic acid bacteria) are made before and during fermentation at 96 h. For each analysis, 10 g of each type of flour were sampled. The experiment is repeated three times and for each analysis three tests were carried out, the average of the tests is considered. The flow diagram of flour is shown in Figure 1.

Physico-chemical analysis

Thirty grams (30g) of corn flour were homogenized in 30 ml of distilled water. For the pH measurement, the pH meter electrode (CONSORT P107) was immersed in the suspension of flour. The pH was read after stabilization of the value on the apparatus screen. Titratable acidity was determined by the method used by Kimaryo et al. (2000) and performed on 10 ml of the previously prepared suspension in the presence of phenolphthalein. Two tests were performed and the average of tests was considered.

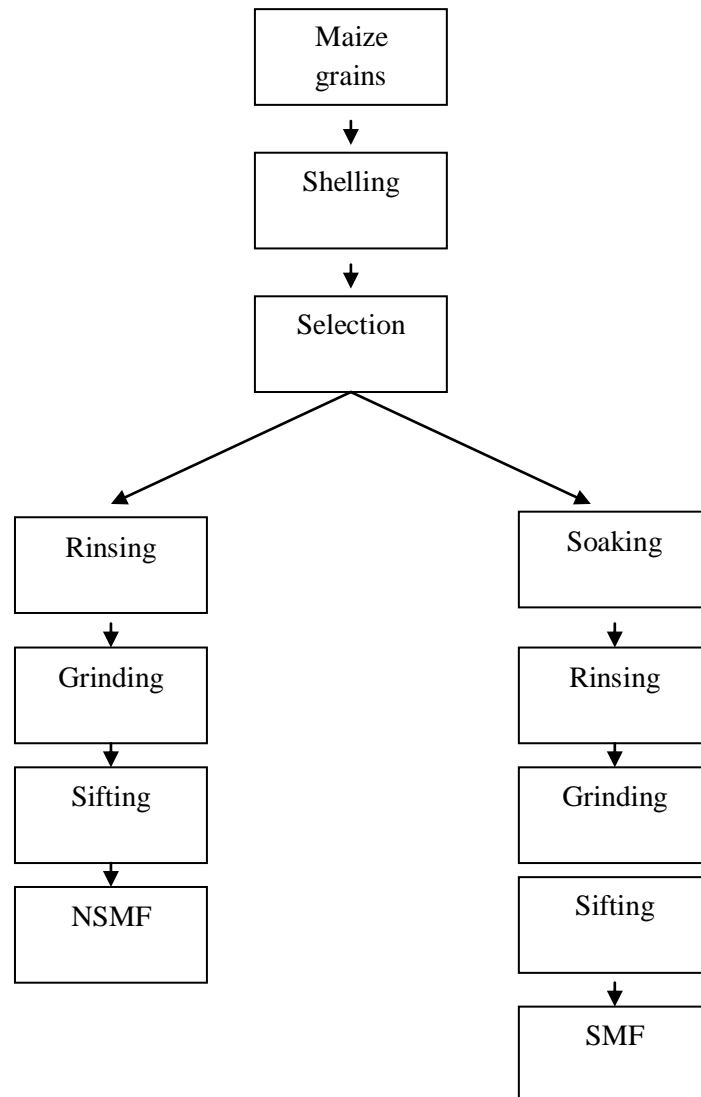


Figure 1. Flow diagram of SMF and NSMF. (1) NSMF: Flour resulted from not soaked maize seeds; (2) SMF: Flour resulted from soaked maize seeds

Preparation of culture medium

Bacterial colony was obtained on solid culture media obtained by mixture of 100 g flour in 1 L of water in a beaker. The mixture is stirred and then decanted for 1 h. The supernatant obtained was centrifuged at speed of 4000 trs/min for 30 min. Resulted solution is sterilized in autoclave at 121°C for 15 min.

Numeration of lactic acid bacteria

In order to obtain the colony suspension, the assessment

of the level of lactic acid bacteria contamination was done by weighting aseptically and transferring 10 g of corn flour from each sample in 90 ml of sterile buffered peptone solution. Samples were thoroughly homogenized and mixed in the stomacher for 5 min to obtain 10^{-1} stock. Further 10-fold serial dilutions were prepared in sterile water up to 10^{-8} . Lactic acid bacteria colonies were counted according to standard AFNOR NF V08-052 on the medium MRS agar (Man Rogosa Sharp agar). Thus, the volume of 0.1 ml of the colony suspension or dilution selected was deposited on the surface of solidified MRS agar into petri dishes. Inoculum was then spread using a sterile glass spreader. The petri dishes containing cultures

were incubated for 48 h at 30°C in an anaerobic jar. All boxes containing between 15 to 150 colonies were used to calculate the number of Colony Formant Unity (CFU) of lactic bacteria per gram of corn flour.

Morphological and biochemical characterization of lactic acid bacteria

The identification of the lactic acid bacterial colonies was based both on morphological and biochemical characteristics such as the production of catalase. This identification was performed firstly on transplanting the enumerated colony exit of the enumeration on MRS medium and secondly on the other hand by Gram coloration test and the test of catalase production. The morphological characterization was done by the differential Gram coloration. This technique give some information on the shape of the cells and allows arranging them in the two great taxonomic groups which are Gram positive bacteria (Gram+) and the Gram negative bacteria (Gram-). For this, a water drop was deposited on a dried and clean glass slide. And then, using the sterile loop, a portion of well-isolated colony on nutrient agar was removed and deposited on the blade while a circular motion so as to obtain a homogeneous thin smear. The resulting smear was fixed above the burner flame before being dipped in gentian violet. After a minute, it was rinsed with water and covered with iodine for one minute. It was then rinsed with water, and then covered with 70% ethanol for 10 seconds. The slide was rinsed with water, and then mounted on microscope immersion objective $\times 100$ for observing the color, morphology and mode of grouping of studied bacteria. The biochemical identification was related to the study of the metabolism and thus the enzymatic equipment. In the specific case of the lactic bacteria, the catalase production test is preferentially required. For its description, a hydrogen peroxide drop was placed on a clean blade and a portion of pure colony was deposited in this drop. The appearance of bubble translates the production of catalase activity.

Evaluation of power acidifying stocks isolated

The objective of this part of the work is to select the group of colonies showing the best profile. The evaluation of the acidifying power of the stocks was made in a colony containing germinated corn grain.

Acidifying power of the isolates

Isolated lactic acid bacteria strains were used for the fermentation of porridge from flour resulted from germinated maize seeds. Each strain was inoculated in

2 ml of porridge and incubated for 24 h at 37°C. At the end of incubation, 30 ml of sterile porridge was inoculated with resulted inoculums. The acidity rate was measured by interval of four hours during 24 h. Changes in total acidity were evaluated and lactic acid bacteria were classified in different groups based on the level of the power of acidification.

Statistical analysis

The statistical exploitation of the results was carried out with the software Statistica (99th edition) at 5% significance level, ANOVA post-test used is that of Duncan.

RESULTS

Changes in pH and titratable acidity of fermenting maize flours (SMF and NSMF).

During the fermentation the pH values decreased whereas titratable acidity increased in both types of flour but the differences between the two flours were not significant. Indeed, Figure 2 shows that the titratable acidity increased from 0.31 to 0.44% and from 0.34 to 0.48% for NSMF and SMF respectively; and moreover, the pH dropped from 4.64 to 3.18 for NSMF and 3.83 to 3 for SMF.

Changes in the average population of lactic acid bacteria in SMF and NSMF

Lactic acid bacteria population recorded in two types of corn flours increased gradually until the third day of fermentation. In fact, this population varied from 7.1 to 8.24 CFU/ml for NSMF and 7.9 to 8.33 CFU/ml for SMF (Figure 3). If this relative growth was significant for NSMF, the same phenomenon was not observed for SMF.

Changes in morphological groups during fermentation process

During the first three days of fermentation of corn flours, the lactic acid bacteria population in shapes of hulls increased in NSMF while the population of bacteria in shape of bacilli decreased in the two types of flours. Indeed, the proportion of cocci increased by 31.63 to 89.76% for NSMF and 62.89 to 88.44% for SMF in the contrary the bacilli decreased from 68.36 to 10.23% for NSMF and 37 to 11.55% for SMF (Figure 4).

Changes in acidification capacity of the isolated lactic acid bacteria strains

The number of groups of bacterial colonies capable to influence the titratable acidity increased until the eighth hour of fermentation (Figure 5). It increased from 3 to 15

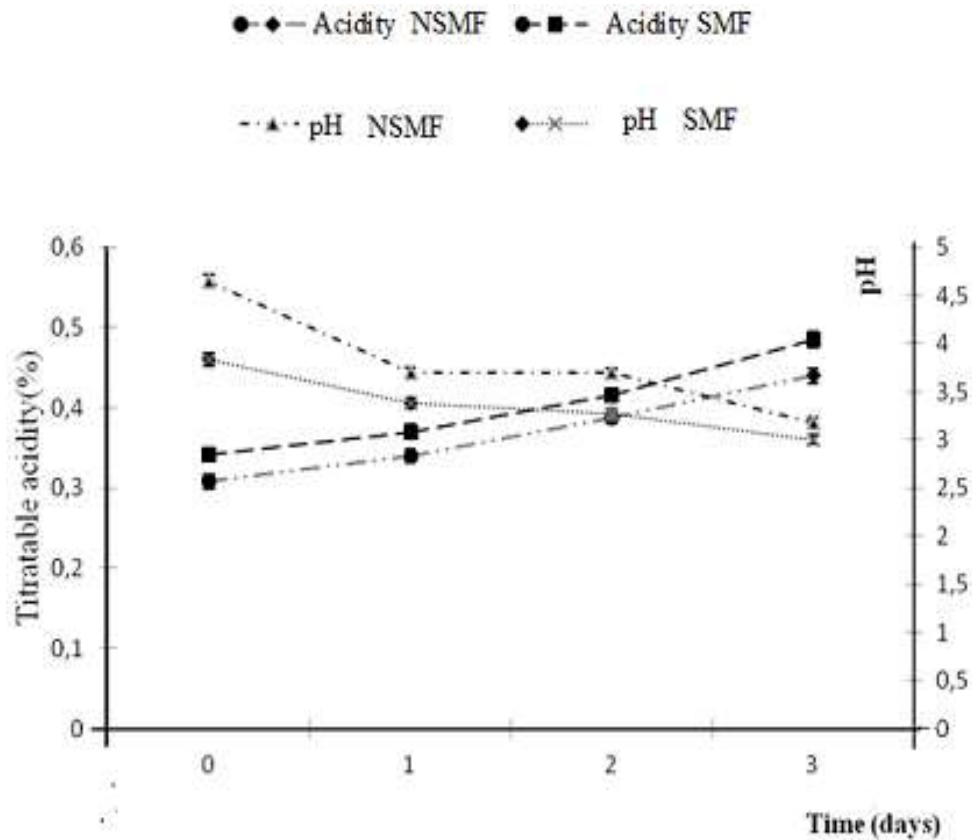


Figure 2. Changes in pH and titratable acidity rate in flour resulted from soaked maize seeds (SMF) and flour obtained from not soaked maize seeds (NSMF).

groups respectively at fourth and eighth hours of fermentation. From the twelfth hour to twenty-fourth hour of fermentation this number dropped notably from 13 to 4.

At fourth hour of fermentation, there are three groups of bacterial strains which might have caused the variation of titratable acidity. The first group induced the rate of acidity variation about 0.09%, the second group caused a rate of variation near 0.04%, and the last group was responsible of a variation around 0.33%. The numbers of each group are respectively 2 colonies for the first group, 26 colonies for the second group and 2 colonies for the third group. At eighth hour of fermentation, there are 15 groups whose numbers vary between 1 and 4. Changes in titratable acidity of these groups are between 0.02 and 0.33%. At twelfth hour of fermentation, there are 13 groups of 1 to 6 colonies with variations of acidity going from 0.2 to 0.44%. At sixteenth hour of fermentation, changes in titratable acidity were ranged between 0.33% and 0.45%. The number of 11 groups varies from 2 to 9. At twentieth hour of fermentation, there are 10 groups with a rate of acidity is between 0.35 and 0.46%. The number of these 10 groups varied from 1 to 9. At twenty-fourth hour of fermentation changes in titratable acidity were comprised between 0.37 and 0.46%. The number of 5 groups varies

between 2 to 14.

Classification of lactic acid bacteria isolated groups involved in maize flour's fermentation

The thirty colonies were all acidified broth corn germinated culture at rates less variable during 24 h of fermentation. Every 4 h of analysis the number of colonies according to the titratable acidity variation is given. Groups of colonies were made according to the minimum change of acidification observed during the twenty-fourth hour of fermentation. This variation were about 0.37%. Thus, at the fourth and eighth hours of fermentation isolate did not vary titratable acidity at a higher rate to 0.37%. At twelfth hour of fermentation, 22 isolates presented a variation of titratable acidity higher than 0.37%. At sixteenth hour of fermentation, 26 colonies showed a rate of acidification higher than 0.37% either 4 new isolates that constituted a second group. At twentieth hour of fermentation, three new colonies presented a variation of titratable acidity higher than 0.37%, they incorporated the third group. Finally at twenty-fourth hour of fermentation, only one colony shows an acidification rate higher than 0.37%, it's the

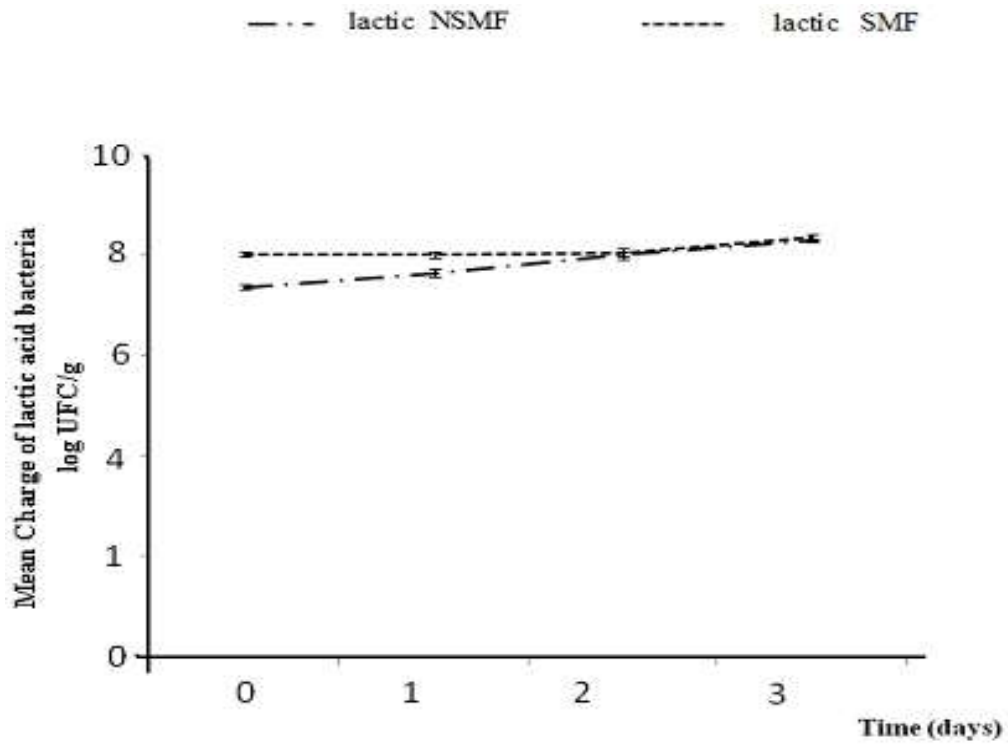


Figure 3. Evolution of mean population of lactic acid bacteria of two types of corn flours: NSMF and SMF.

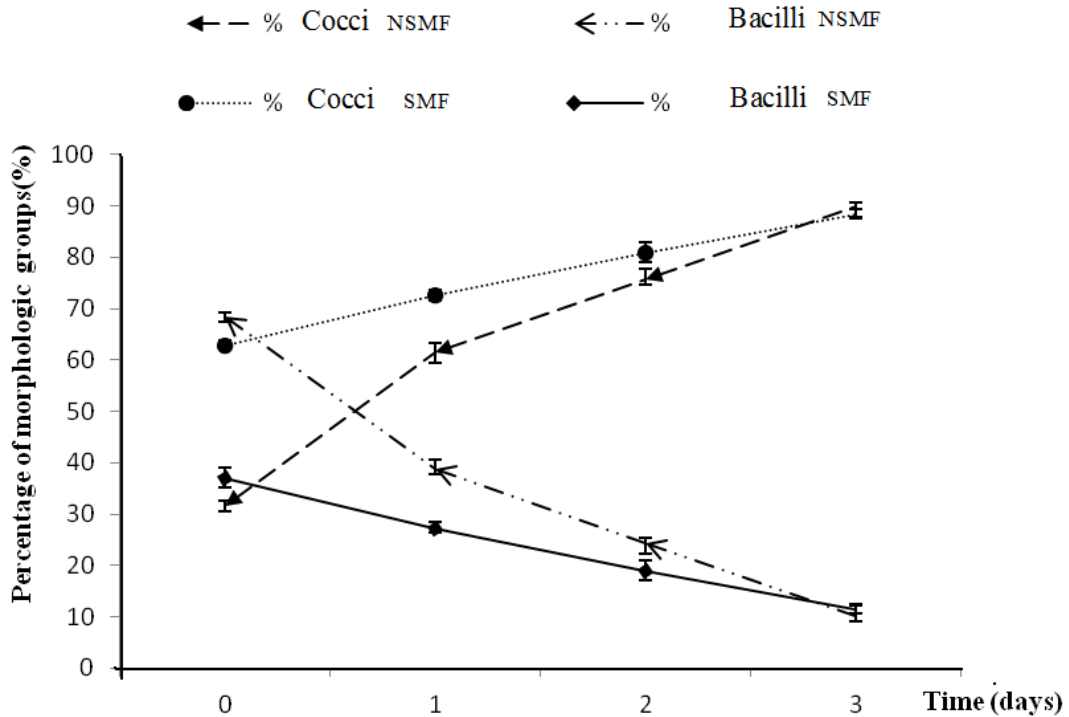


Figure 4. Changes in lactic acid bacteria morphological populations in NSMF and SMF.

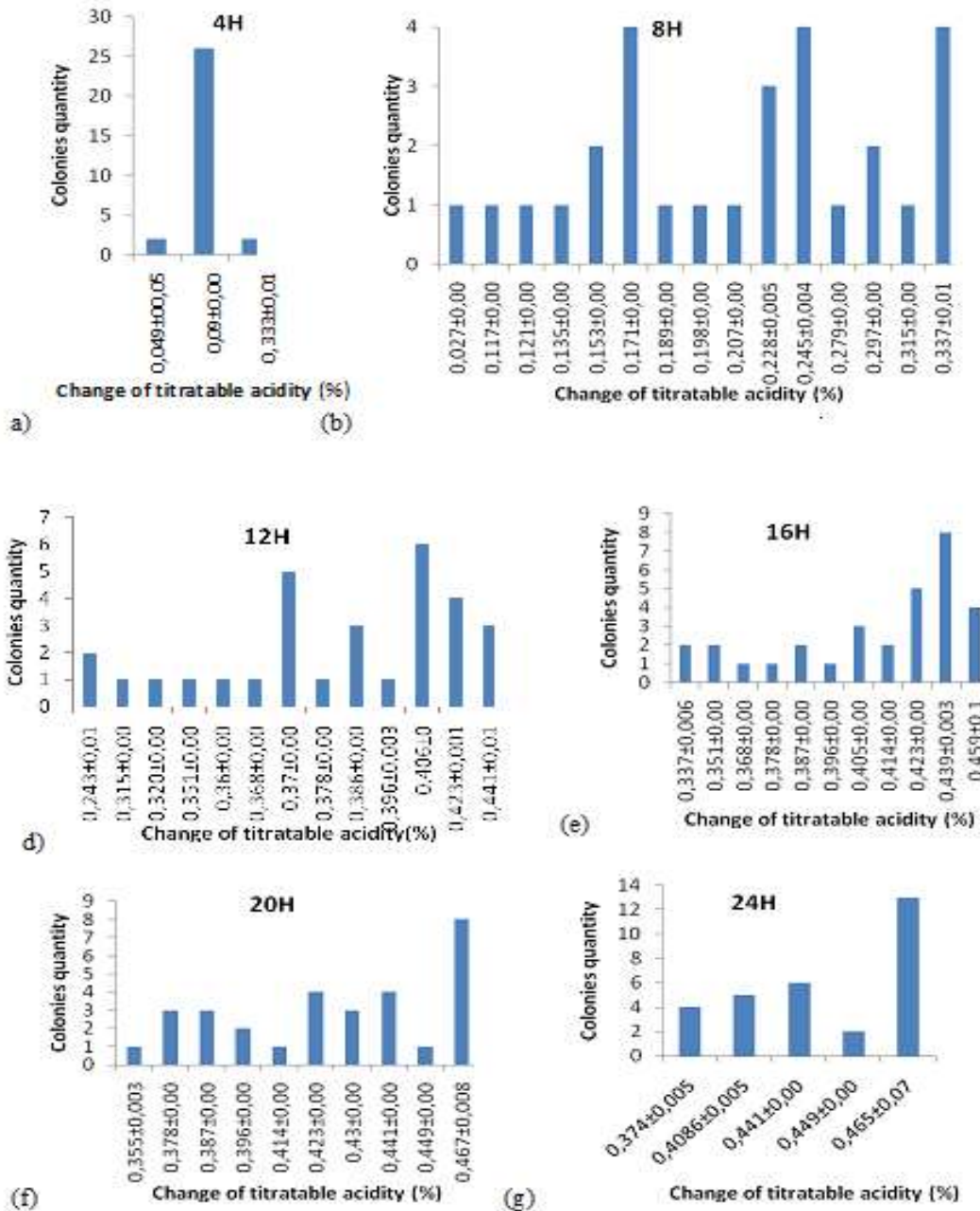


Figure 5. Distribution of isolated lactic acid bacteria according to their acidification capacity during wild fermentation of maize flours.

fourth group. The groups were ranked from 1 to 4. The percentage of colonies in each group was determined. Thus group 1 was the most acidifying colony with 76.7% of colonies. Then there were group 2, respectively with 13, 33% of colonies, the group 3 with 6.67% of the colonies, and group 4 with 3.33% of the colonies (Figure 6).

The acidification kinetics of each group followed for 24 h of fermentation showed that the titratable acidity rates of each group increased during the fermentation of maize medium. But from Gupta test, the result was that only the colonies group 1 had a change of acidity

significantly higher than others and showed that these colonies have the best acidification profile.

DISCUSSION

Lactic fermentation is a very old method of food preservation. It presents the advantage to improve at the same time hygienic property and organoleptic quality of some foodstuffs. Acidification produced during this process is an essential parameter for improving the medical quality

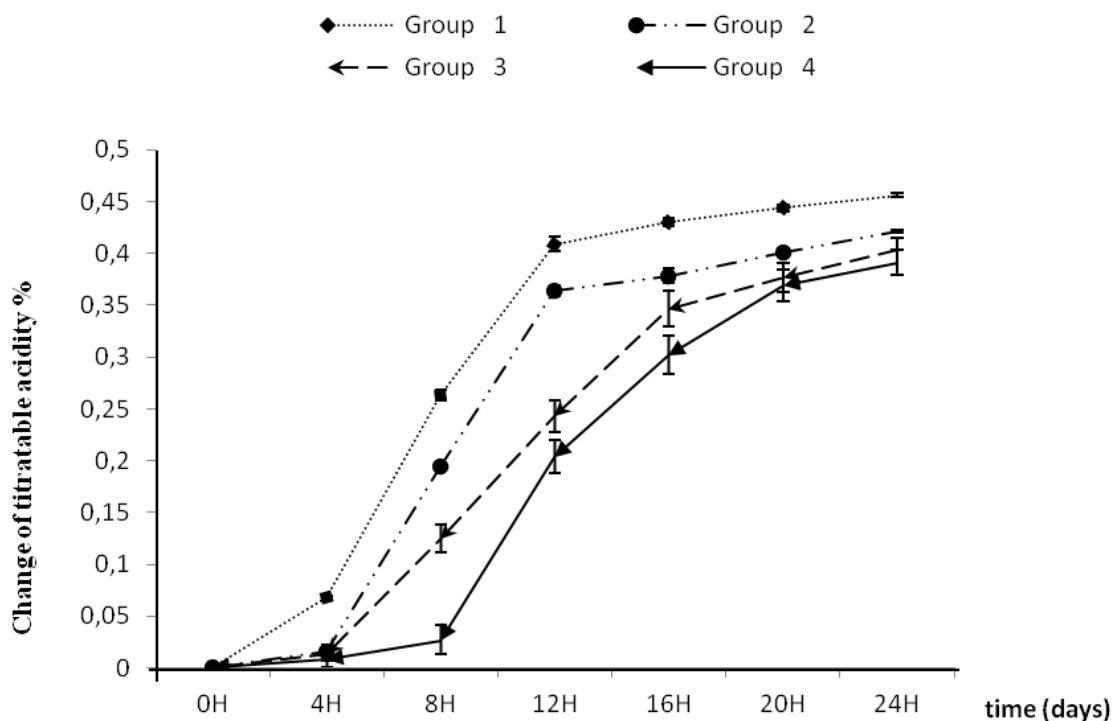


Figure 6. Kinetics of acidification due to the lactic acid bacteria groups involved in spontaneous fermentation of corn flour.

of food. The purpose of this study was to optimize the acidification of maize flour by fermentation. Thus, the physico-chemical and microbiological analyses were carried out on two types of three days fermented corn flours.

These tests were conducted to study the effect of soaking of maize grains on corn flour acidification. Obtained results showed a variation of pH, titratable acidity and population of lactic acid bacteria. Moreover, the acidifying power of thirty colonies of isolated lactic acid bacteria was also evaluated.

At the start of fermentation, physico-chemical characteristics including pH and titratable acidity of the corn flour made from soaked maize (SMF) recorded a more significantly acidic than the not soaked maize flour (NSMF). This result revealed that the soaking of maize grains influences the acidification of maize flour. This acidification is due to the fermentation that occurs during soaking of grain. During the soaking, proliferation conditions of lactic acid bacteria population such anaerobiosis and presence of nutrients were created. And then resulted organic acids occurred and acidify flour (Mugula et al., 2003). These results confirmed those of Trèche et al., (1992) who observed a relatively high acidity of porridge made from soaked maize seeds. This acidification of SMF at the start of fermentation reduces health risks during various heading. Indeed, acidic environments are unfavorable to the growth of

certain harmful germs such as *Clostridium botulinum* which produces botulinic toxin, a mortal toxin for the human adult (Fleming and McFeter, 1981). Thus at the beginning of fermentation, health quality of SMF is potentially higher than that of NSMF.

Furthermore, the acidity parameters such as pH and titratable acidity of the two flours indicated that they were acidified significantly during three days of fermentation. These results indicated that fermentation improves maize flour acidity. This decrease in pH correlated with the increase of titratable acidity is due to the action of lactic acid bacteria by production of organic acids during their intense life activity (Nche et al., 1994). Assouhoun (2008) got similar results on the maize paste in fermentation. Lactic acid bacteria acidify the medium in which they develop and help to reduce the health risk associated with microbial contamination of flour. Most of pathogens are inhibited at low pH (Caplice and Fitzgerald, 1999). In addition, studies conducted by Bressani (1972) have shown that maize porridges are considered bland by consumers who often resort to addition of acid ingredient such as lemon. The acidification produced by fermentation contributes to improve the organoleptic quality of maize meal.

The acidity of SMF was higher than those of NSMF. But the changes in the acidity parameters of two flours during fermentation were not significantly different. So,

higher acidity recorded in SMF than that in NSMF during the whole fermentation process was due to its initial acidity rate resulted from the soaking of corn seeds. And soaking of grains provided pre-acidification conditions to maize meal and predisposed to have a higher acidity at the end of fermentation process.

At the start of fermentation process, important population of lactic acid bacteria isolated from SMF comparatively to NSMF was due to the previous growth of lactic acid bacteria during the soaking of seeds. Soaking of the grains favored lactic fermentation by creation of environmental conditions such as absence of oxygen and pH low inducing so fast lactic acid bacteria multiplication (Mensah, 2000).

Lactic acid bacteria charge of NSMF increased significantly during the fermentation. This increase was due to the tolerance of these bacteria to the lactic acid compounds and their ability to use the substrates present in the medium for fermentation process (Mugula et al., 2003). Lactic acid bacteria population of SMF did not increase significantly because the pH conditions and secreted bacteriocins were selected from the fermentation processing during soaking of grains.

Lactic acid bacteria produced several organic acids which penetrate in the cell wall of most pathogenic microorganisms and inhibit their reproduction. This bacteriostatic effect can reduce the health risk associated to microbial contamination of these flours and improve food security of consumers (Cotter and Hill, 2003). Therefore SMF has a better stability compared to NSMF. The different tests have also shown a preponderance of cocci compared to the bacilli in both types of flour. This prevalence of cocci would translate that the lactic bacteria most acidifying in the fermentation medium are shaped like shells. Certain lactic hulls such as *Leuconostoc œunos* and *Lactococcus lactis* are recognized for their high acidifying power. *Lactococcus lactis* is able to produce in 3 h a quantity of organic acid higher than 1.6 g/L, which inhibits the growth of *Escherichia coli* a main cause of morbid diarrhea affecting children in developing countries (Caplice and Fitzgerald, 1999). The thirty colonies of isolated lactic acid bacteria were all able to acidify the germinated maize medium. But the acidification capacity is not the same. Thus, four formed groups were classified according to their acidifying power. This classification was used to select the group having the most acidifying lactic acid bacteria. The colonies in this group were able to produce in 8 h of fermentation acid levels higher than 0.35% which is the minimum inhibitory concentration of *Clostridium botulinum* (Cotter and Hill, 2003). Moreover with these same quantities of acid, Odunfa and Adeyele (1985) observed an inhibition of *Escherichia coli* growth and reduction of the *Shigella* population in the Nigerian fermented such as drink, 'Ogi'. Therefore, these colonies could be used after their identification in the starter culture preparation to ferment maize flour and help to reduce health risks to consumers. In addition studies carried

out by Mensah (1985) have shown that use of starter culture for fermentation of infant flours made it possible to reduce the risk of mal-nutrition and diarrhea, which are the main causes of infant mortality in developing countries.

Conclusion

This study aimed to evaluate the effect of soaking of the seeds on the acidification of corn flour by fermentation and the acidifying power of lactic acid bacteria isolated from resulted corn flour to improve the acidification process of flour during the fermentation. Corn flour SMF showed higher acidification rate in the start and at the end of fermentation process. Thus, for an optimal rate of acidification, grains must undergo a pre-soaking. Selection of lactic acid bacteria according to their acidifying power has enabled to establish 4 groups of bacteria. Among these groups, only one group presents the best profile of acidification. The use of colonies in this group to carry out the fermentation of maize flour will make it possible to optimize the acidification of that latter.

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