The microflora of fermented nixtamalized corn

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Short communication

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Received 1 March 2002; received in revised form 1 December 2002; accepted 15 February 2003

Abstract

Nixtamalization is a traditional process that improves the nutritional quality of corn. To provide a means of utilizing the nutritional benefits of nixtamalized corn and improve product acceptability, lactic acid fermentation was applied. The objective of the study was to study the microbial profile and establish the important lactobacilli of fermenting nixtamalized corn dough. Two batches of cleaned whole corn were subjected to the process of nixtamalization, using two concentrations of lime (0.5 or 1.0%), milled, made into a dough (50% moisture) and fermented spontaneously for 72 h. A control sample was prepared without alkaline treatment. pH and titratable acidity of the dough were measured. Aerobic mesophiles, lactic acid bacteria, yeasts and molds were enumerated on Plate Count Agar (PCA), deMan, Rogossa and Sharpe (MRS) Agar and Malt Extract Agar (MEA), respectively. The identity of lactobacilli present was established at the species level using API 50 CHL. The pH of all the fermenting systems decreased with fermentation time with concomitant increase in titratable acidity. Lactic acid bacteria in numbers of $1.6 \times 10^9$, $2.3 \times 10^9$ and $1.8 \times 10^9$ cfu/g, respectively yeasts and molds, and numbers of $8.0 \times 10^7$, $5.0 \times 10^5$ and $1.7 \times 10^5$ cfu/g, respectively were observed in the control and the two nixtamalized (0.5% and 1.0% lime) samples after 48 h of fermentation. Lactobacilli identified in the fermenting nixtamalized corn dough were Lactobacillus plantarum, Lactobacillus fermentum and Lactobacillus cellobiosus as well as Pediococcus spp. The study demonstrates that nixtamalized corn though alkaline in nature can be subjected to spontaneous fermentation to produce a sour product.

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Keywords: Microflora; Fermentation; Nixtamalization; Corn; Corn dough

1. Introduction

Corn (Zea mays) is one of the main staples in West Africa, and it is vital to the survival and food security of a large segment of the population contributing over 55% of the total energy intake (Sefa-Dedeh and Mensah, 1989). It is consumed in several forms following operations such as soaking, milling, fermentation, boiling and packaging (Sefa-Dedeh, 1993). Corn is deficient in the vitamin niacin and contains a low amount of protein which is deficient in lysine and tryptophan. However, it contains a reasonably fair amount of sulphur-containing amino acids and is high in leucine and aromatic amino acids. There has been several attempts at improving the protein quality of corn-based diets (Akpadunam and Sefa-Dedeh, 1995; Afoakwa, 1996; Sefa-Dedeh et al., 2000). The strategy has been to add a high-protein material such as legumes.

Fermentation of corn dough has been reported to improve the safety by inhibiting Shigella and enter-
Enterotoxigenic *Escherichia coli* (Mensah et al., 1991). They reported that maize dough that has been fermented for 3 days delayed growth of *Shigella* and enterotoxigenic *E. coli* (ETECs). There is therefore considerable evidence that lactic acid fermentation inhibits the survival and multiplication of a number of bacterial pathogens. The antimicrobial properties of fermented foods appear to be their most interesting quality.

The process of nixtamalization in combination with other processes can be applied to improve the processing and utilization of maize. Sefa-Dedeh (1993) reported that nixtamalized corn when subjected to material solid-state fermentation showed a reduction in pH and cooked paste viscosity. The process of nixtamalization has been reported to yield several nutritional improvements including the faster release of amino acids, decrease in tannin levels of high tannin grain, increase in the bioavailability of iron and other minerals, increase in free calcium levels, increase in free nicotinic acid and increase in availability of niacin (Hulse et al., 1980; Vivas et al., 1987; Bharati and Vaidehi, 1989). This paper examines the microflora of fermented nixtamalized corn.

2. Materials and methods

2.1. Preparation of corn dough

Corn was cleaned, soaked in water for 24 h and milled (Disc Attrition Mill Model 10-2A, New Delhi, India). The meal was made into a 50% moisture dough and fermented for a period of 72 h. Nixtamalized fermented corn dough was prepared by boiling corn in lime (0.5% or 1%) for 30 min each. The boiled corn was soaked in the cooking liquor for 14 h, washed with water and milled using a disc attrition mill (Model 10-2A). The meal (masa) was made into a dough of about 65% moisture by adding a pre-determined amount of water. The nixtamalized corn dough was fermented for a period of 72 h.

2.2. Microbiological analyses

The method of Amoa-Awua et al. (1997) was used to prepare the dough and steep water samples for microbiological analyses. Enumeration of aerobic mesophiles was carried out on Plate Count Agar (PCA, Merck 5463, Darmstadt, Germany) incubated at 30 °C for 3 days; lactic acid bacteria enumerated on deMan, Rogossa and Sharpe (MRS) Agar (MRS, Merck 10660) were incubated anaerobically in an anaerobic jar at 30 °C for 5 days; yeasts and molds were counted on Malt Extract Agar (MEA, Merck 5398) containing 100 mg chloramphenicol (Chloramphenicol Selective Supplement Oxoid) and 50 mg Chlortetracycline (Sigma C-4881, St. Louis, MO, USA) per litre and incubated at 25 °C for 5 days. The determinations were done in triplicates and the mean values recorded.

2.3. Identification of lactic acid bacteria

All colonies were subcultured in MRS broth medium (MRS, Fluka 69966, Buchs, Switzerland) and streaks onto MRS agar substrate until pure cultures were obtained. MRS isolates were examined by colony and cell morphology, Gram reaction and catalase production. *Lactobacillus* spp. was recognized as Gram-positive and catalase-negative rods. They were further sub-grouped based on detailed examination of their cell morphology which was accomplished by comparing high ($\times$ 100) magnification fields of the cells in a microscope (Model BH-2, Olympus, Japan). The identity of *Lactobacilli* was established at the species level by examining representative isolates for utilization of 49 carbohydrates using API 50 CHL (BioMerieux, Marcy-L'Etoile, France) according to the manufactures instruction. The inoculated strips were incubated anaerobically at 30 °C for 72 h and read after 24, 48 and 72 h of incubation. The lactobacilli were identified using an API 50 CHL identification table. The Gram-positive, catalase-negative cocci were tentatively identified by cell morphology.

2.4. Determination of pH and titratable acidity

The pH of the steep water was determined at the beginning and the end of the steeping period. About 100 ml of the steep water was centrifuged at 3000 rpm for 15 min using a MSE, Mistral 3000i centrifuge (Model MBS 300, Sanyo, UK). The pH of the supernatant was measured using a Lab pH meter (Model PHM 92, Radiometer, Copenhagen). For determina-
tion of pH during fermentation, 10 g of dough was mixed with 100 ml of CO₂-free distilled water. The mixture was allowed to stand for 15 min, shaken at 5-min intervals and centrifuged at 3000 rpm for 15 min using a centrifuge (Model MSB 300). The supernatant was decanted and pH measured using a Lab meter (Model PHM 92). Ten (10)-millilitre aliquots (triplicates) were pipetted and titrated against 0.1 M NaOH to 1% phenolphthalein end point. Acidity was calculated as gram lactic acid/100 g sample. The determinations were done in triplicates and the mean value recorded.

3. Results and discussion

3.1. pH

The pH of the steep water for the corn samples soaked in water without alkaline treatment was 6.68, and this fell to 4.63 after 24 h of steeping period (Table 1). This reduction of pH is attributed to the onset of fermentation during soaking. The steep water for corn nixtamalized with 0.5% and 1% lime was very high (11.67 and 11.81, respectively), and there was no reduction of pH with steeping time. The boiling of the corn might have contributed to the observed stable high pH. The pH of the non-nixtamalized dough decreased from 4.98 to 3.54 following 72 h of fermentation (Table 1). The reduction in pH has been reported to be due to the production of acids by fermenting microorganisms (Sefa-Dedeh, 1993; Akpapunam and Sefa-Dedeh, 1995). The dough prepared from nixtamalized corn had an initial alkaline pH of 8.0–9.7. When subjected to fermentation, the nixtamalized dough also showed a reduction in pH. The differences in the pH of the two types of dough suggest production of an equal amount of acid in all the samples; however, in the nixtamalized samples, some of the acids produced were required to neutralize the alkali thus leading to a high final pH and low acidity.

3.2. Microbial population during steeping of corn and fermentation of dough

3.2.1. Lactic acid bacteria

Enumeration of lactic acid bacteria showed that the steep water for the non-nixtamalized corn contained $5.2 \times 10^7$ cfu/g at the beginning of steeping. This increased to $4.5 \times 10^7$ cfu/g after 24 h. On the contrary, the steep water from the nixtamalized samples showed no growth for lactic acid bacteria. The boiling process could have eliminated the bacteria from the samples and therefore prevented the onset of fermentation. The corn dough samples showed characteristic changes in pH with fermentation. At the beginning of fermentation, it was found that the non-nixtamalized samples had $3.6 \times 10^7$ cfu/g of lactic acid bacteria (Table 2). The nixtamalized samples contained $1.3 \times 10^6$ and $1.2 \times 10^6$ cfu/g of lactic acid bacteria for 0.5% and 1% lime, respectively. After 24 h of fermentation, it appeared that the population of lactic acid bacteria was relatively higher in the nixtamalized samples than the control (Table 2). The presence of Ca²⁺ ions could have stimulated the growth of lactic acid bacteria. By the end of the 72 h of fermentation, the concentrations of lactic acid bacteria were fairly uniform in all the samples. The high pH of the nixtamalized samples did not seem to adversely affect the growth and activity of lactic acid bacteria in the fermenting dough samples.

3.2.2. Aerobic mesophiles

Enumeration of aerobic mesophiles in the steep water on Plate Count Agar (PCA) showed that a few was able to withstand the process of nixtamalization (Table 3). These counts were very low and also surviving aerobic mesophiles did not appear to have been able to multiply significantly during steeping of lime-treated maize. Increases in the population of aerobic mesophiles were observed during the fermentation of both the nixtamalized and non-nixtamalized doughs. Examination of their colonies and cell morphologies showed that substantial proportions of these were similar to the lactic acid bacteria enumerated on

Table 1
pH of the steep water and nixtamalized dough during fermentation

<table>
<thead>
<tr>
<th>Percent lime</th>
<th>Steep water Steeping time (h)</th>
<th>Dough Fermentation time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 24</td>
<td>0 24 48 72</td>
</tr>
<tr>
<td>0</td>
<td>6.68 4.63</td>
<td>4.98 3.72 3.62 3.54</td>
</tr>
<tr>
<td>0.5</td>
<td>11.67 11.52</td>
<td>8.04 4.35 3.87 3.73</td>
</tr>
<tr>
<td>1.0</td>
<td>11.81 11.95</td>
<td>9.74 5.04 4.32 4.10</td>
</tr>
</tbody>
</table>
MRS. Lactic acid bacteria are often microaerophilic and able to grow on PCA.

3.2.3. Yeasts and molds

Yeasts and molds appeared not to be able to survive the process of nixtamalization as seen in Table 4. However, as in the case of the lactic acid bacteria, substantial combination of the nixtamalized corn with yeasts and molds occurred during the subsequent operations of milling and kneading before the dough fermentation. Thus, whereas no growths were obtained on malt agar plates of the 0.5% and 1% nixtamalized corn during steeping, counts in the order of $10^5$ cfu/g were obtained in the nixtamalized doughs at the start of dough fermentation. In the control (non-nixtamalized) dough, a hundred-fold increase in yeast counts was observed during the 72 h of fermentation; however, in the nixtamalized dough, the yeast counts remained constant throughout the period of fermentation.

3.3. Characterization and identification of the species of the dominant lactic acid bacteria

Examination of the cell morphology of the final lactic acid bacteria population of both the 0.5% and 1% lime-treated doughs showed five groups of lactic acid bacteria. The first group consisted of rods occurring in pairs and chains of varying lengths. A lot of the pairs were joined at an angle with light shimmering around them as seen under the phase contrast microscope. The second group consisted of short rods in singles, pairs and chains of varying lengths. The third group was made up of long rods with few occurring in singles and a lot of pairs and chains of varying lengths. All the first three groups consisting of Gram-positive, catalase-negative rods were tentatively identified as *Lactobacillus* species. The fourth group was cocci which occurred in singles, pairs and chains of varying lengths. The last group was cocci occurring mostly in pairs and fours with a few as single or aggregate cells and was tentatively identified as *Pediococcus* spp.

The dominant strain of *Lactobacillus* present in the fermenting nixtamalized corn (0.5 and 1% lime) utilized L-arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, mannitol, sorbitol, D-mannoside, N-acetyl glucosamine, amygdaline, arbutine, salicine, cellulbiose, maltose, lactose, melibiose, saccharose, trehalose, melezitose, D-raffinose, β-gentiobiose, D-turanose and gluconate, and was identified as *Lactobacillus plantarum*.

From the nixtamalized corn prepared with 0.5% lime, a total of 26 cultures isolated from the dough

### Table 2
Population of lactic acid bacteria* (cfu/g)

<table>
<thead>
<tr>
<th>Percent lime</th>
<th>Steep water</th>
<th>Dough</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steeping time (h)</td>
<td>Fermentation time (h)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>0</td>
<td>$5.2 \times 10^5$</td>
<td>$4.5 \times 10^7$</td>
</tr>
<tr>
<td>0.5</td>
<td>$&lt;1.0 \times 10^2$</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>$&lt;1.0 \times 10^2$</td>
<td>0</td>
</tr>
</tbody>
</table>

* Enumerated on MRS Agar, reflecting Gram-positive, catalase-negative rods and cocci.

### Table 3
Population of aerobic mesophiles* (cfu/g)

<table>
<thead>
<tr>
<th>Percent lime</th>
<th>Sleep water</th>
<th>Dough</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steeping time (h)</td>
<td>Fermentation time (h)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>0</td>
<td>$4.0 \times 10^4$</td>
<td>$8.7 \times 10^7$</td>
</tr>
<tr>
<td>0.5</td>
<td>$1.1 \times 10^2$</td>
<td>$2.0 \times 10^3$</td>
</tr>
<tr>
<td>1.0</td>
<td>$1.2 \times 10^3$</td>
<td>$6.3 \times 10^2$</td>
</tr>
</tbody>
</table>

* Enumerated on PCA, including Gram-positive and Gram-negative bacteria.
were identified. Fourteen (14) isolates representing 54% of the cultures were *L. plantarum*. The second strain of *Lactobacillus* spp. found in relatively high amounts in the fermenting nixtamalized corn utilized L-arabinose, D-xylose, galactose, D-glucose, D-fructose, maltose, melibiose, saccharose and D-raffinose. This was tentatively identified to be *Lactobacillus fermentum*, and was found only in the nixtamalized corn prepared from 0.5% lime.

*Lactobacillus cellobiosus* was the third group of lactobacilli identified in the nixtamalized corn produced with 0.5% lime. It utilized L-arabinose, ribose, D-xylose, galactose, D-glucose, D-mannose, salicine, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, D-raffinose and β-gentiobiose. Two (2) isolates of this strain representing 8% of the lactobacilli were found.

The fourth group was Gram-positive, catalase-negative cocci which represented 4% of the cultures isolated. The fifth and last group of isolates found in the fermenting nixtamalized corn prepared with 0.5% lime were *Pediococcus* spp., which were identified by tetrad formation.

The dominant lactic acid bacteria isolated from the fermented nixtamalized corn prepared with 1% lime were identified to be *Pediococcus* spp., representing 88% of the microflora present. One (1) isolate of *L. plantarum* representing 6% of the total number was identified. The other isolates representing 6% of the microflora present were cocci (unidentified). These were detected at levels of $10^5$ cfu/g.

*Pediococcus* spp. and *Pediococcus* spp. were detected at levels of $10^8$ and $10^9$ cfu/g in both nixtamalized corn (0.5 and 1% lime), and were the dominating microorganisms found during the fermentation of nixtamalized corn. These results tally with the other researcher on fermenting corn dough. *Nche et al. (1994)* reported that *L. plantarum* and *Pediococcus* spp. dominate the latter stages of corn dough fermentation. *L. fermentum* has been reported to play a dominant role in fermented corn dough (*Halm et al., 1993*). Their presence in the fermenting nixtamalized corn is therefore not unusual. *L. plantarum* and *Pediococcus* spp. were present in both nixtamalized corn. However, *L. plantarum* was the dominant microorganism in the nixtamalized corn prepared with 0.5% lime while the *Pediococcus* spp. dominated the nixtamalized corn prepared with 1% lime. These observations suggest the possibility of some microbial succession that is dependent on the concentration of lime in the fermenting corn.

**Acknowledgements**

This study was funded through the Bean–Cowpea Collaborative Research Support Program by the United States Agency for International Development Grant No. DAN-1310-G-SS-6008-00.

**References**


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**Table 4**

<table>
<thead>
<tr>
<th>Percent lime</th>
<th>Steep water</th>
<th>Dough</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slopping time (h)</td>
<td>Fermentation time (h)</td>
<td>Slopping time (h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>$9 \times 10^3$</td>
<td>$1.7 \times 10^5$</td>
</tr>
<tr>
<td>0.5</td>
<td>$&lt;1.0 \times 10^2$</td>
<td>$&lt;1.0 \times 10^5$</td>
</tr>
<tr>
<td>1.0</td>
<td>$3.0 \times 10^5$</td>
<td>$&lt;1.0 \times 10^2$</td>
</tr>
</tbody>
</table>

* Enumerated on Malt Extract Agar, supplementing with Chloramphenicol and Chlorotetacycline.
biological and aromatic characteristics of fermented maize doughs for kenkey production in Ghana. International Journal of Food Microbiology 19, 135–143.


