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Chemical and Rheological Description of Pozol Dough Fermentation Inoculated with Streptococcus Infantarius Subsp. Infantarius 25124 and Lactobacillus Plantarum A6

A. Yolotli Téllez-Díaz, Gloria Díaz-Ruiz, Mariana Ramírez-Gilly, Carmen Wacher, Alberto Tecante*

Departamento de Alimentos y Biotecnología, Facultad de Química, Universidad Nacional Autónoma de México, Cd. Universitaria, CdMx, 04510, México

Abstract

Pozol is a refreshing beverage prepared with fermented dough made from nixtamalized maize. Its microbiota has been studied, but little is known about the biophysical modifications involved in fermentation. The dough was inoculated separately with the amylolytic lactic acid bacteria *Streptococcus infantarius* subsp. *infantarius* 25124 and *Lactobacillus plantarum* A6 and changes in the microbial growth, pH, moisture content, the concentration of reducing sugars, residual starch, and viscoelasticity were monitored during fermentation. The purpose was to study the way changes occur, their time evolution, and interrelation. The fermented dough exhibited practically the same viscoelastic behavior regardless of the inoculated strain and fermentation time. Changes in the chemical indicators were consistent with microbial growth, i.e., pH and starch concentration decreased significantly in comparison with an inoculum-free control during the first five days. Moisture content remained essentially constant, while the level of reducing sugars increased moderately. The intensity of these changes revealed differences in the properties of the fermented 1 doughs attributed to the activity of the different strains. However, modifications were congruent with the behavior of the doughs

Keywords: Fermentation, Maize, Pozol, Starch, Viscoelasticity

Corresponding author: **Alberto Tecante**

Departamento de Alimentos y Biotecnología, Facultad de Química, Universidad Nacional Autónoma de México, Cd. Universitaria, CdMx, 04510, MÉXICO

Email: mailto:tecante@unam.mx

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Introduction

Pozol is an acidic and refreshing beverage, prepared from naturally fermented nixtamalized maize dough dispersed in water. Other typical ingredients may include salt, sugar, honey, cocoa powder, or dried chili peppers (Ulloa, Herrera & Lappe, 1987). Lactic acid bacteria (LAB) are responsible for 90 to 97 % of the total activity of the fermenting microbiota in pozol dough. The genus Streptococcus accounts for 20 to 50 % of the microbiota. Apparently, starch is first degraded by LAB amylases, and the resulting sugars and lactate become substrates for a secondary microbiota. Also, when the pozol dough is shaped into balls, the overall activity is more important on the periphery. However, the lactic acid bacteria growth occurs mainly at the center of the pozol balls (Ampe, Omar, Moizan, Wacher & Guyot 1999). Unlike other non-nixtamalized maize and starchy fermented foods, where *Lactobacillus plantarum* strains predominate (Kalui, Mathara, Kutima, Kiiyukia & Wongo, 2009; Obinna-Echem, Kuri & Beal, 2014), *Streptococcus bovis*, later identified as Streptococcus infantarius subsp. infantarius (Sii 25124) (Díaz-Ruiz, Guyot, Ruiz-Terán, Morlon-Guyot & Wacher-Rodarte, 2003) has been found to be the dominant species in *pozol dough*. *Lactobacillus* spp. have been isolated from pozol, but as they are scanty amylolytic, *Lactobacillus plantarum* strain A6 (*L. plantarum* A6), a highly amylolytic strain isolated from fermented cassava was used (Giraud, Champailler & Raimbault, 1994). This strain can degrade cassava raw starch depending on whether the pH of the culture medium is controlled. At constant pH 6.0 a high amylolytic activity was observed, but when pH was not controlled low amylolytic 2 activity resulted (Giraud et al., 1994). Also, non-amylolytic species can use malto-oligosaccharides accumulated during fermentation as an

energy source (Díaz-Ruiz et al., 2003).

On the other hand, the cultural importance and inherent complexity of pozol justify the study of the reproduction of amylolytic microorganisms, starch degradation, sugars production, and changes in the viscoelastic properties of the dough. This approach will provide essential knowledge about how these modifications operate and the time scale of their occurrence. The concentration of mono and disaccharides in maize is markedly reduced during nixtamalization. Therefore, starch becomes the primarily available source for microbial growth along fermentation. Thus, it is reasonable to postulate that the rheological characteristics of the dough might be affected by starch degradation and production of short-chain carbohydrates during fermentation, with the consequent modification of its viscoelasticity. This work aimed to study the individual effect of the amylolytic activity of *L plantarum* A6 and *Sii* 25124, on the chemical changes and viscoelastic properties of fermented pozol dough and to determine the differences between the impact of the amylolytic activity of these bacteria. The results will add further insight on the physicochemical properties of this complex system.

Materials and methods

Dough sterilization

The pozol dough prepared from nixtamalized maize was collected at the Pino Suárez Market in the city of Villahermosa, in the Mexican State of Tabasco. The fresh dough was refrigerated for six hours and frozen at -20 °C until further use. Three kilograms of thawed dough were weighed in a plastic bag, and its moisture content was adjusted to 36 % with distilled water by kneading inside the container. Then, the dough was pressed uniformly with a spatula and divided into portions of ca. 500 g. Each piece was placed in a plastic bag, flattened to a thickness of about 2 cm to expel 3 air and reduce the amount of oxygen, and the container was thermally sealed. The dough pouches were sterilized by exposition to 25 kGy of gamma radiation for 78 h.

Inoculum preparation and dough inoculation

The dough was inoculated separately with *Sii* 25124, an amylolytic microorganism isolated from pozol, and *L. plantarum* A6, a highly amylolytic organism, isolated from fermented cassava. They were handled and kept in glass beads with glycerol at -80 °C according to reported procedures (Nagel & Lawrence, 1972). A glass bead of each strain was placed in two separate test tubes containing 10 mL of APT broth (BD DifcoTM) in a laminar flow hood. The tubes were shaken in a vortex (Genie 2, Fisher Scientific) and incubated 24 h at 30 °C. Subsequently, 0.1 mL of culture was taken from each tube and inoculated separately in 10 mL of APT broth. The inocula were incubated 18 h at 30 °C. Then, 0.1 mL of each culture was inoculated again separately in 10 mL of APT broth and incubated 18 h at 30 °C.

The final culture of each strain was centrifuged at 10000 rpm (J2-21 M / E, Beckman, USA) for 10 min, resuspended in sterile deionized water and 0.1 mL of each one inoculated again in 10 mL of APT broth. They were incubated 18 h at 30 °C. The purity and number of colony-forming units (CFU) in the suspension were determined from Gram staining and plate counting, respectively, following standard procedures. The surface of the plastic bag containing the sterile dough was disinfected with 70 % ethanol and opened with a flamed spatula inside the laminar flow hood. The necessary volume of the resuspended inoculum in sterile deionized water was added to obtain 106 CFU/g of wet mass for each microorganism. The dough inside the bag was kneaded from the periphery to the center.

Fermentation

The dough was placed in glass jars covered with aluminum foil fastened with an elastic rubber band, previously sterilized at 121 °C for 15 min. The dough was divided into portions of ca. 100 g. Each part was transferred with a sterile spatula to a jar, compacted with a flamed metal rod, pressed to eliminate cracks in the dough, and covered with sterile agar to ensure similar conditions and anaerobiosis. The jars were covered with the same aluminum foil, and band with which they were sterilized, and incubated at 30 °C for twenty-four days.

All the procedures were done under aseptic conditions. Controls for each sampling time were prepared and handled in the same way and added with sterile deionized water instead of inoculum. The microbiological activity of the controls was determined by plate count (CFU/g) in PCA (BD DifcoTM), MRS (BD DifcoTM) and MRS with 1.5 % soluble potato starch instead of glucose (MRSA) media. The absence of growth indicated lack of contamination of the dough by externalmicroorganisms and confirmed its sterility.

Sample preparation and microbiological analysis

A sample was always taken from the center of the dough with a sterile spatula. A portion of 10 g was added to 90 mL of 0.1 % peptone and homogenized for one minute at normal speed (Stomacher® 400, Seward, Blender Laboratories). Decimal dilutions were made with the same diluent. 0.5 mL of the sample was mixed with 4.5 mL of 0.1 % peptone-water and shaken in a vortex. Viable bacteria were determined by plate counts in the different culture media; PCA for aerobic mesophilic bacteria, MRS for lactic acid bacteria, and MRSA for amylolytic lactic acid bacteria. For each medium, 0.1 mL of the three appropriate dilutions of the sample were placed in a Petri dish, respectively, spreading it with a sterilized glass rod and incubated at 30 ° C for 48 h. Microbial growth was observed and quantified. For lactic acid bacteria, Gram positive and catalase 5 negative tests were confirmed in a representative sample of colonies. Contaminating bacteria were determined from PCA medium, when in the same Petri dish, by separate counts of colonies larger than 1 mm in diameter. Likewise, colonies with a diameter less than or equal to 1 mm, for which a representative sample was catalase negative, were considered. These colonies were counted as lactic acid bacteria.

Fermentation indicators

pH

A portion of 5 g of dough was dispersed in 10 mL of deionized water under sterile conditions in a sterilized bag and homogenized in a Stomacher at normal speed for 60 s. pH was measured in a glass-electrode potentiometer (3020 pH Meter, Jenway) previously calibrated with pH 4 and 7 buffers.

Moisture content

Two grams of dough were placed in constant-weight glass capsules and dehydrated at 120 °C in an oven (Precision, Thermo Electron Corporation, USA) until constant mass. Then, the dehydrated samples were put into a desiccator, and the moisture content was calculated by mass difference.

Reducing sugars

A portion of 10 g of dough was dispersed in 90 mL of sterile deionized water and homogenized in a Stomacher at normal speed for 60 s. The sample was filtered and diluted with water to 100 mL in a volumetric flask. A 1 mL aliquot was mixed with 1 mL of DNS reactant, and heated for 5 min by immersion in boiling water in a bath (Polystat, Cole-Parmer). The solution was cooled and diluted with 10 mL of distilled water. Absorbance was read at 540 nm in a spectrophotometer 6 (Génesis 10 UV, Thermo Electron Corporation) against a reagents blank treated like the sample. Reducing sugars were calculated by reference to a standard curve of glucose in the range of 0.2-2 mg/mL. This curve was obtained with absorbances at 540 nm obtained from a 2 mg/mL glucose stock solution.

Residual starch

A piece of 50 to 250 mg of dry sample was mixed with 4 mL of water in a test tube. Starch was gelatinized heating the container 15 min in a boiling water bath. The tube was cooled to room temperature, and 3 mL of 72 % perchloric acid solution was added rapidly under stirring. The sample was stirred with a glass rod for 1 min, and the procedure repeated for 15 to 20 min. The rod was rinsed with 20 mL of water, and washes were collected in the tube. The dispersion was centrifuged (J2-21M/E Beckman, USA) at 1000 rpm for 5 min and the supernatant was separated by decantation. The residue was again extracted with 4 mL of water and 3 mL of perchloric acid. The supernatants were mixed and diluted with water in a 50 mL volumetric flask.

The amount of starch was determined with the STA20 kit (Sigma-Aldrich, Mexico), based on enzymatic digestion with amylase and amyloglucosidase. Here, starch in the aqueous medium is hydrolyzed to glucose by -amylase and amyloglucosidase. Then, glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. The latter reacts with o-dianisidine in the presence of peroxidase and forms a brown complex. O-dianisidine reacts with sulfuric acid and yields a more stable pink color. Its intensity is measured at 540 nm and is proportional to the original concentration of glucose.

Rheometry

Each dough portion was examined in serrated parallel plates 25 cm in diameter and 3 mm gap in a commercial rheometer (ARES RFS III, TA Instruments, NJ, USA). First, the zone of linear viscoelasticity (ZLV) was determined by a strain sweep at an angular frequency of 6.28 rad/s. Then, a frequency sweep was run at a constant strain within the ZLV, from 0.1-100 rad/s. The variation of the dynamic moduli with frequency was obtained. *Statistical analysis* Results are presented as the mean of three replicates with the corresponding standard deviation. A one-way ANOVA was carried out to find the differences between experimental data with a significance level (α) of 0.05 combined with a Tukey´s range test.

Results and discussion

Control dough: Fermentation indicators and rheological behavior

No significant ($p > 0.05$) changes between zero and twenty-days of incubation were observed in pH, moisture content, reducing sugars and residual starch of the control media (Table 1).These results confirmed the absence of microbiological activity. The dough showed the typical behavior of a deformable elastic-viscous paste with a high solid-to-liquid proportion (Fig. 1). G' was always superior to G" with a slight dependence on frequency; n in the expression $G' \alpha \omega^n$ had an average value of 0.0678 with a standard deviation of 0.00180 over the entire fermentation time of twenty-four days. Therefore, the elastic nature of the dough remained unchanged with incubation time. The same happened with G". Consequently, the variation of the phase angle, along the frequency range was between 6 to 11 degrees he loss angle of a purely elastic solid is zero degrees. Then, the behavior of pozol dough was dominated by the elastic character. These results served as a reference to those obtained with *Sii* 25124 or *L. plantarum* A6.

Analysis	Value
pH	5.47 ± 0.038
Moisture (%)	36.7 ± 0.386
Reducing sugars (mg/mL)	0.925 ± 0.000189
Residual starch (%)	3.05 ± 0.0309

Table 1: Chemical analyses of the control dough.

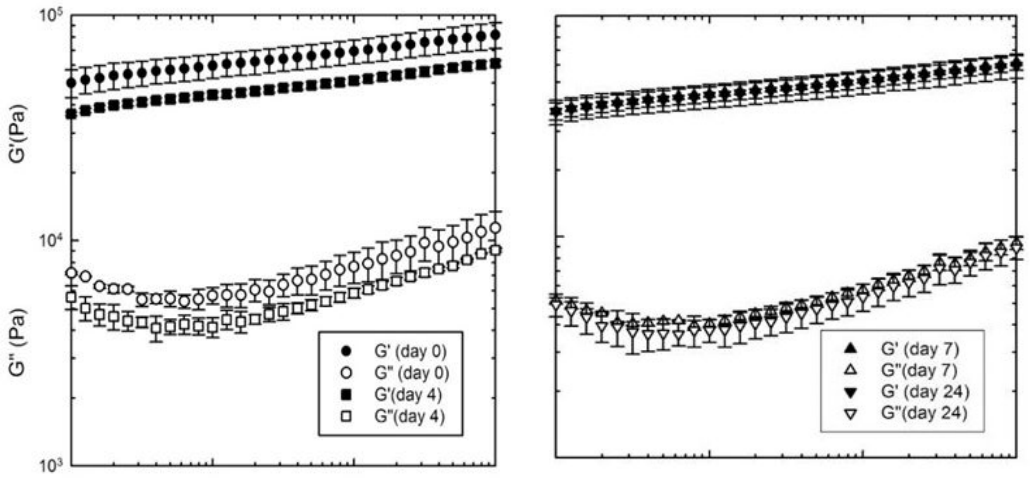


Fig. 1: Variation of dynamic moduli (top) and tan (bottom) with frequency at 25 °C and 0.1 %strain, for the control dough at different fermentation times

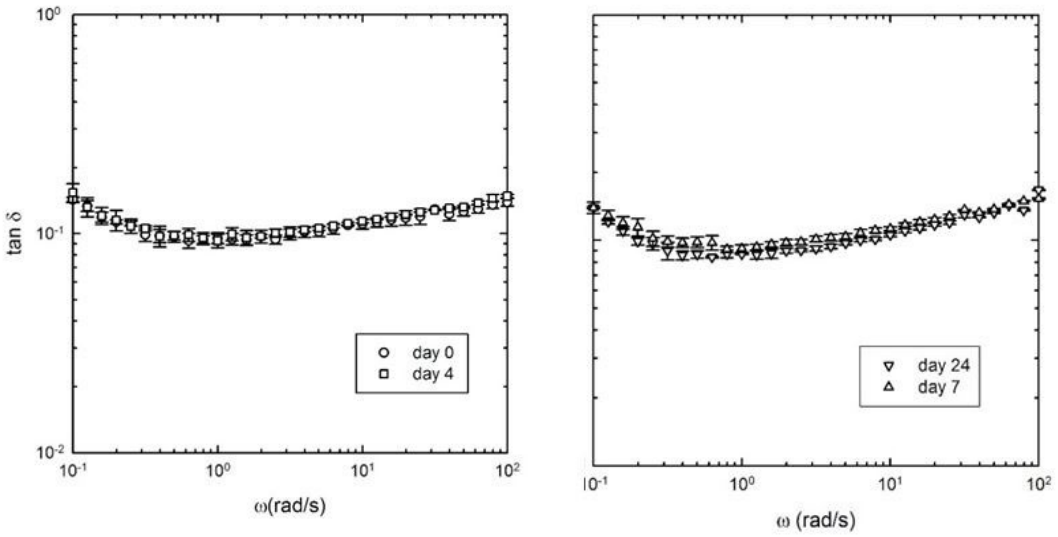


Fig. 1: Variation of dynamic moduli (top) and tan (bottom) with frequency at 25 °C and 0.1 %strain, for the control dough at different fermentation times

Doughs inoculated with *Sii* 25124 and *L. plantarum* A6

Microbial growth

The growth of *Sii* 25124 at 30 °C was similar in the PCA, MRS, and MRSA culture media (Fig. 2:left). The exponential phase occurred in the first nine days, and the stationary zone in the last ten days. This behavior can be attributed to the low tolerance of this strain to acidic pH because as fermentation proceeded the pH dropped to 4.3 (see Fig. 4: top).

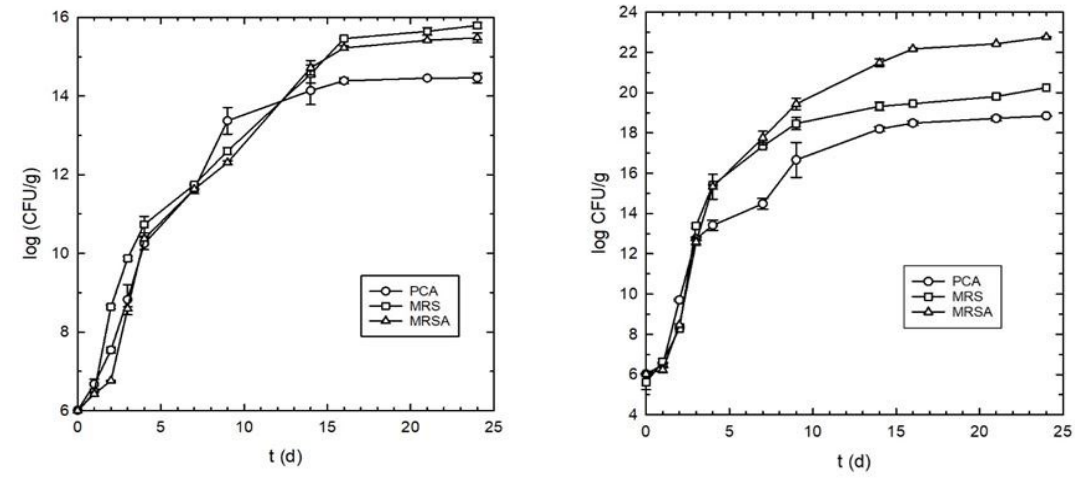


Fig. 2: Growth of *Sii* 25124 (left) and *L. plantarum* A6 (right) at 30 °C in different culture media. Continuous lines are included as a guide.

The growth of *L. plantarum* A6 was intense in the first five days of fermentation (Fig. 2: right). After this period the population increased and reached the quasi-stationary phase at sixteen days of fermentation. The microorganism reproduced better in the MRSA medium than in the MRS and PCA. In addition to the intense growth in the first five days, *L. plantarum* A6 continued to form colonies until the last day presumably because it could hydrolyze starch and survive and develop in the medium. The activity of α-amylase depends strongly on pH when this microorganism is grown in a synthetic culture with cassava starch, either raw or heat-treated, as the only carbon source. A high amylase activity was observed for pH 6.0; 45 g of raw starch produced 41 g of lacticacid in three days of fermentation. In contrast, no decrease in starch concentration was observed when pH was not controlled (Giraud et al., 1994). The situation was different when the microorganism was grown in pozol dough. The experimental microbial growth data were fitted to the logistic and Gompertz models given by equations (1), and (2), respectively (Zwietering, Jongenburger, Rombouts, & van't Riet, 1990).

$$ln\left[\frac{N(t)}{N_0}\right] = \frac{A}{1+exp\left[\frac{4\mu_m}{A}(\lambda-t)+2\right]} \quad (1)$$

$$ln\left[\frac{N(t)}{N_0}\right] = Aexp\left\{-exp\left[\frac{e\mu_m}{A}(\lambda - t) + 1\right]\right\} \quad (2)$$

These empirical expressions contain three regression parameters; A, the asymptotic value ln[N∞/N0], the time lag, and m, the maximum specific growth rate of the microorganism. Likewise, N(t) and No are the instantaneous and initial number of cells, respectively, and e is a constant given by e^1 = 2.718. These expressions have proven useful to describe the growth of twenty-seven different microorganisms, among them L. plantarum (Zwietering et al., 1990). The logistic and Gompertz models produced good fittings, but the latter was retained because it produced the lowest sum of squared residuals SSR = $\sum_i(y_{exp} - y_{model})^{i^2}$ where y_{exp} and y_{model} are the experimental and predicted values of ln[N(t)/No], respectively. Fig. 3 (left) shows the predictions of equation (2) for *Sii* 25124 in the PCA (top), MRS (middle), and MRSA (bottom) media. The symbols are experimental data, and the dashed lines show the predictions for the twenty-four days of fermentation. Fig 3 (right) shows the fitting of the experimental data for *L. plantarum* A6 with the same equation for the three media in the same order as for *Sii* 25124. The values of m and goodness-of-fit parameters t and SSR for all the experimental data are shown in Table 2. Regressions were good (r > 0.98) and the model fits the data on the whole fermentation time. However, r and SSR values were better for *Sii* 25124. The maximum specific growth rate in PCA was 12, and 17 % higher than in MRS and MRSA, respectively, while the difference between MRS and MRSA was only 4 %. This behavior confirms the similarity observed in Fig. 2 for *Sii* 25124. *L. plantarum* A6 showed higher maximum specific grow rates confirming the trends shown in Fig. 2. The m of *L. plantarum* in PCA was 61 % higher than that of *Sii* 25124.

	***Sii* 25124**		***L. plantarum* A6**		
PCA					
μ_m(d^{-1})	r	SSR	μ_m(d^{-1})	r	SSR
0.200	0.9961	0.02424	0.322	0.9866	0.1699
MRS					
0.178	0.9905	0.06839	0.490	0.9883	0.2225
MRSA					
0.171	0.9924	0.06015	0.386	0.9903	0.1884

Table 2: Maximum specific growth rate, m, and goodness of fitting parameters for LAB in different culture media predicted by the Gompertz model.

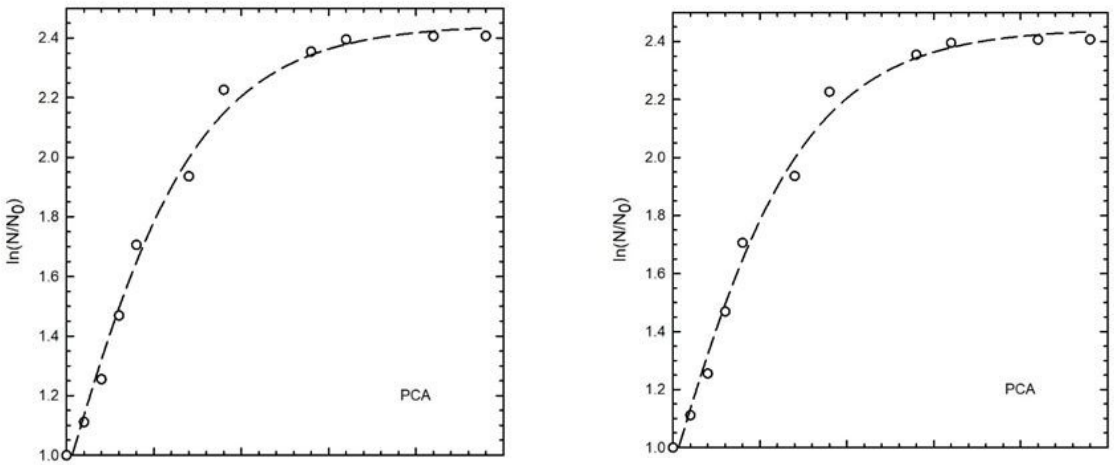


Figure 3: Gompertz model predictions (dashed line) for Sii 25124 (left) and L. plantarum A6 (right) in PCA, MRS, and MTSA media.

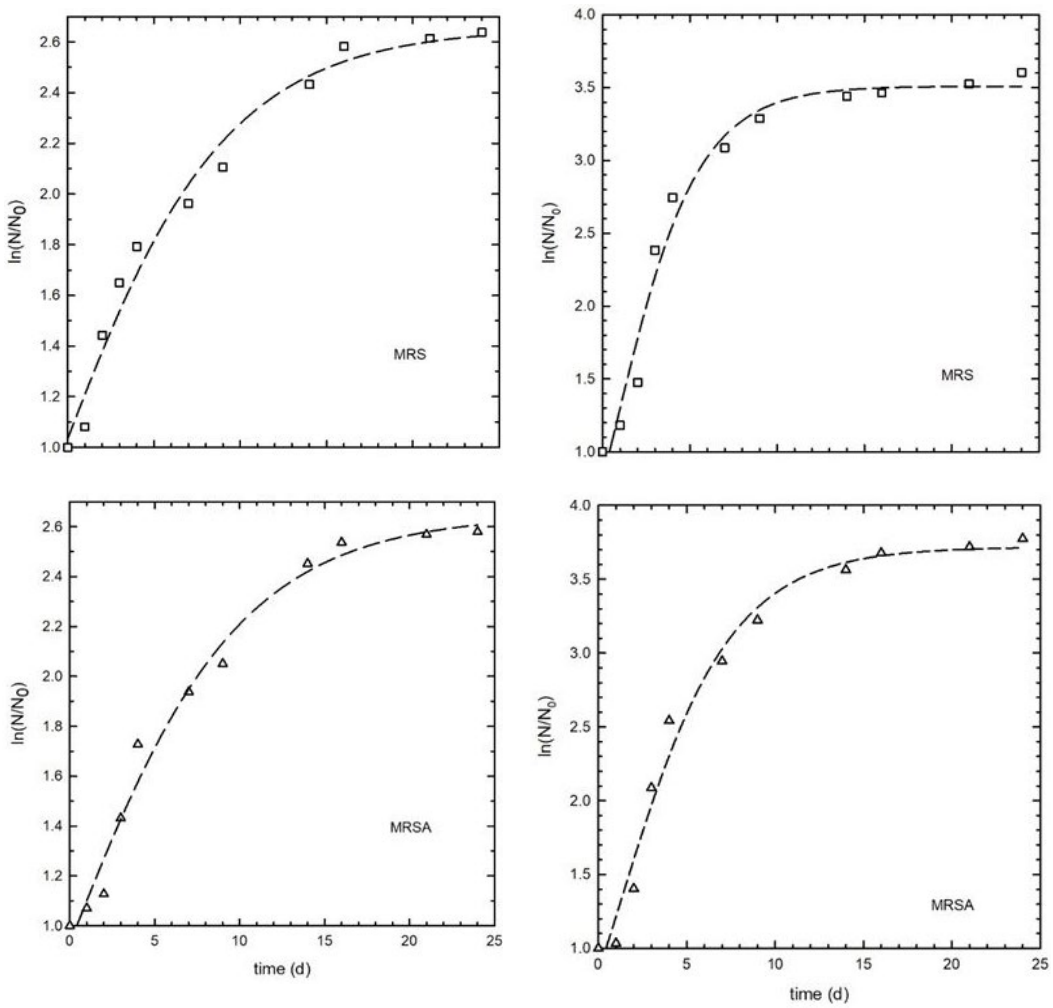


Figure 3: Gompertz model predictions (dashed line) for Sii 25124 (left) and L. plantarum A6 (right) in PCA, MRS, and MTSA media.

The specific rates in MRS and MRSA where 2.8 and 2.3 times greater than those of *Sii* 25124. However, r and SSR values were three to seven times higher. Despite this difference, the Gompertz model adequately correlated the experimental data.

Fermentation indicators

pH

The average pH for *Sii* 25124 dropped from 5.44 to 4.28 with a low standard deviation (< 0.70 %) along the twenty-four days of fermentation (Fig. 4: top). However, the rate of decrease was not constant; it was 0.149 pH units/day during the first four days and then dropped to 0.0288 pH units/day for the rest of the fermentation time.

This behavior suggests a rapid production of acidic species, mainly lactic acid, in the early stage of fermentation followed by a drastic drop. This overall change is consistent with the microbial growth previously discussed. Figure 4 (top) also shows the average pH change of two fermentations for *L. plantarum* A6. The pH decreased from 5.40 ± 0.018 to 3.74 ± 0.255. In the first three days the rate of decrease was 0.559 pH units/day (r = 0.9436). After this period pH remained essentially constant around 3.70. This reduction in pH is almost four times greater and one day shorter than that for *Sii* 25124. This shows the strong acidification activity resulting from carbohydrates transformation into acidic species, e.g. lactic acid. The initial pH is favorable for bacterial growth with a high amylolytic activity carried out by an extracellular α-amylase with an optimum pH of 5.0 (Rodríguez-Sanoja, Morlon-Guyot, Jore, Pintado, Juge & Guyot, 2000).

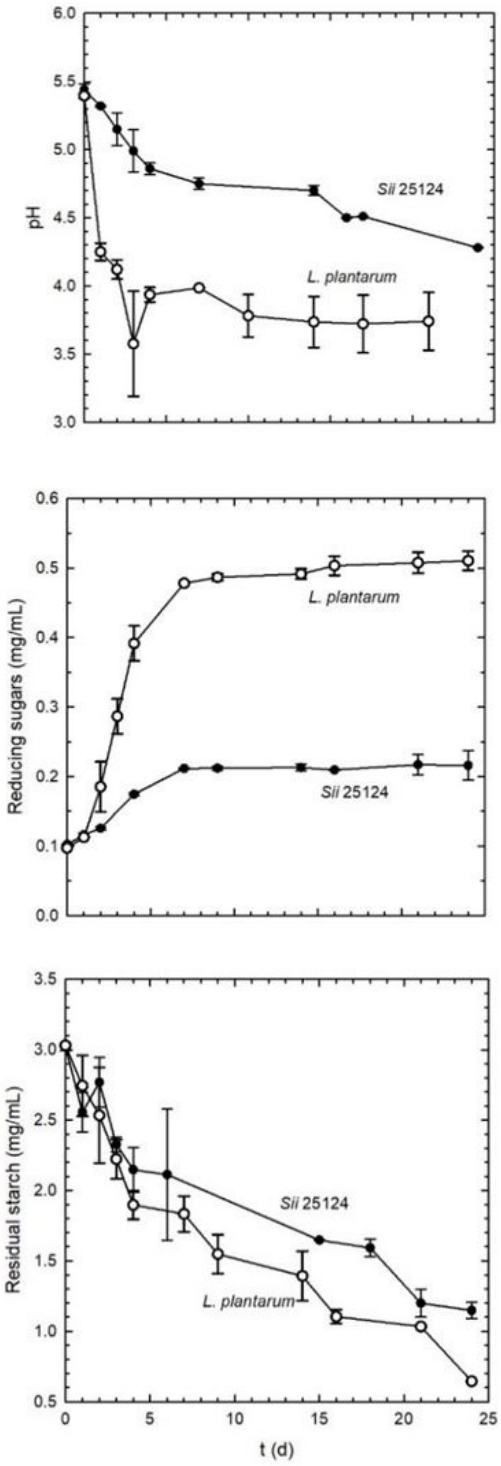


Figure 4: Fermentation indicators for *Sii* 25124 and *L. plantarum* A6 growth at 30 °C. pH (top), reducing sugars concentration (middle), and residual starch (bottom). Values are the mean of two fermentations. Continuous lines are included as a guide.

Moisture content

The average moisture content remained constant at 34.8 2.75 %, and 36.5 0.206 % during fermentation for *Sii* 25124 and *L. plantarum* A6, respectively. This condition rules out any variation in rheological response due to changes in the moisture of the pozol dough and shows that the aqueous environment was favorable for microbial growth.

Reducing sugars

In the first seven days of fermentation with *Sii* 25124, the average concentration of reducing sugars increased linearly (r = 0.9911) from 0.102 to 0.212 mg/mL with standard deviations lower than 0.50 % at a rate of 0.0163 mg/mL.d (Fig. 4: middle). After this period the concentration remained practically constant at around 0.212 to 0.216 mg/mL. This behavior confirms the presence of reducing sugars, e.g. glucose, or short-chain carbohydrates with a reducing end produced presumably by the amylolytic activity of *Sii* 25124. However, apparently these carbon sources were not involved in microbial growth as their concentration did not decrease during the process or they were produced because of the amylolytic activity and consumed at the same rate. Here again, an intense change was observed during the first days of fermentation. The concentration of reducing sugars increased from 0.0972 ± $6.01.10^{-4}$ to 0.392 ± 0.0252 mg/mL at an approximate rate of 0.0763 mg/mL. d (r = 0.9723) during the first four days for *L. plantarum* A6 (Fig. 4: middle). The concentration reached approximately 0.510 mg/mL after the ninth day. This final level of reducing sugars was about two-fold higher than for *Sii* 25124.

Residual starch

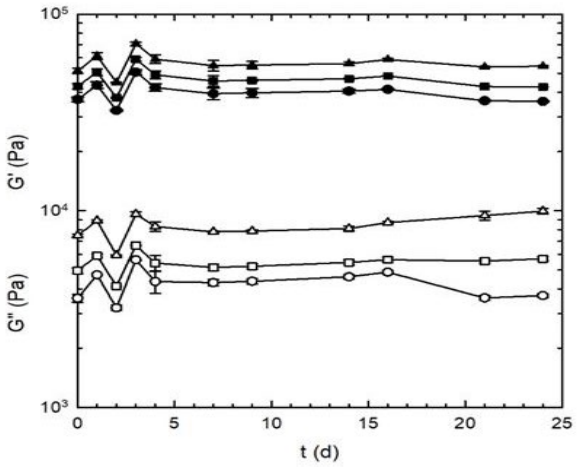
The average residual starch content decreased from 3.02 ± 0.0262 % to 1.15 ± 0.0587 % during the twenty-day period for *Sii* 25124 (Fig. 4: bottom). As it happened with pH and reducing sugars concentration, two different rates were observed; an approximately linear (r = 0.9016) decrease rate of 0.197 %/day in the first four days and 0.0521 %/day (r = 0.9827) for the rest of the process. This change is the result of the amylolytic activity of *Sii* 25124 that was continuous along the entire fermentation period. Again, the decrease was more significant during the first days. The amylolytic capacity of *Sii* 25124 combined with the starch content in the dough resulted in a noticeable degradation of this carbohydrate.

In the case of *L. plantarum* A6 the residual starch content decreased from 3.03 ± $8.45.10^{-3}$ to 0.644 ± $3.00.10^{-3}$ % during the entire fermentation (Figure 4: bottom). The rate of decrease was 0.279 %/day in the first four days (r = 0.9974) and dropped to 0.0602 %/day (r = 0.9785) for the rest of the time. This represents an almost five-fold reduction. Both rates were 29 and 13 % higher than the corresponding for *Sii* 25124. These values show the high amylolytic activity of *L. plantarum* A6 that resulted in more significant starch degradation than *Sii* 25124. The two microorganisms can grow in the presence of starch as the primary substrate. However, *Sii* 25124 has a lower tolerance to acidic pH, and *L. plantarum* A6 is a microorganism with greater amylolytic activity.

Rheological behavior

The variation of the dynamic moduli with frequency for the dough inoculated with *Sii* 25124 was essentially the same of the control dough (Fig. 1). Moreover, the behavior exhibited a non- significant ($p > 0.05$) change with fermentation time; the overall elastic-viscous behavior was not modified. Figure 5 shows the variation with the fermentation time of the dynamic moduli for three 17 different frequencies for two fermentations. In one fermentation (Fig. 5, left), G' and G" show and up-down behavior within the first five days, while in the other one (Fig. 5, right) the moduli decreased within the same period. After this initial lapse, the moduli remained practically unchanged along the rest of fermentation. However, the initial behavior is not characterized by significant changes in the rheological behavior, but it is consistently present in different systems, e.g., when the dough is fermented with the natural microbiota (unpublished results).

These variations in the first days of fermentation coincide with the active cell growth (Fig. 2) and starchdegradation (Fig. 4). Nevertheless, the viscoelastic properties of the dough were substantially constant. This fact can be explained by the considerable proportion of insoluble solids that maintain dough's rigidity even when starch is being degraded, i.e., the material behaves as a particulate system with a high solid to water proportion regardless of starch degradation. In this way, the viscoelastic behavior is governed by the solid particles embedded in a constant moisture environment rather than by the macromolecular nature and composition of the dough, given primarily by the physical state of starch granules. Besides, the elastic-viscous character was not affected either. The behavior of the loss angle of the fermented dough was practically the same of the control shown in Figure 1 (bottom) for the control. The loss angles ranged from 6 to 11 degrees, confirming the solid-like nature of the doughs. The same happened with the dough inoculated with L. plantarum A6. The predominantly elastic character of the doughs was observed from the variation with frequency of the loss angle (data not shown). This angle varied from 5 to 10 degreesalong the entire frequency interval. This response is comparable to that for *Sii* 25124.



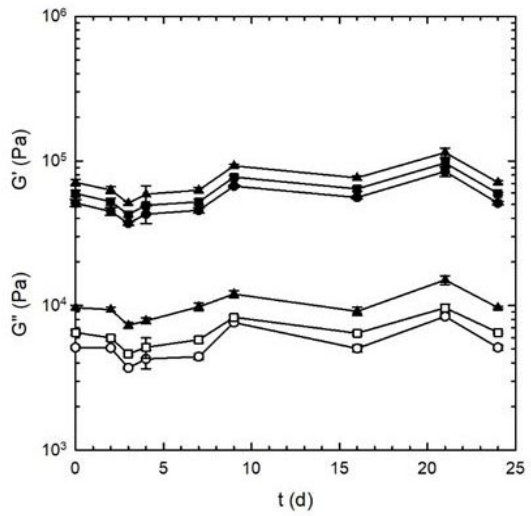


Figure 5: Variation of G' (filled symbols) and G" (void symbols) with fermentation time at 25 °C,0.1 % strain, and 0.631 (circles), 6.31 (squares), 63.1 rad/s (triangles up) for two fermentations of maize dough inoculated with *Sii* 25124

Figure 6 shows the variation of G' and G" with fermentation time for three different frequencies for the doughs inoculated with *L. plantarum* A6. Although both moduli seem to increase with fermentation time for the different frequencies, they were not significantly different ($p > 0.05$) over the entire process for a constant frequency. The chemical transformations associated with the presence of *L. plantarum* A6 are evidenced by the decrease in pH, the reduction in the residual starch content and the increase in the concentration of reducing sugars (Fig.4). Starch in the pozol dough was degraded by the bacteria. Strains of *L. plantarum* A6 isolated from cassava, grow adequately when carbohydrates such as starch or other sugars are found in abundance (Díaz-Ruiz et al. 2003). Nevertheless, contrary to what was expected, the viscoelastic behavior of the inoculated doughs did not exhibit substantial modification with fermentation time.

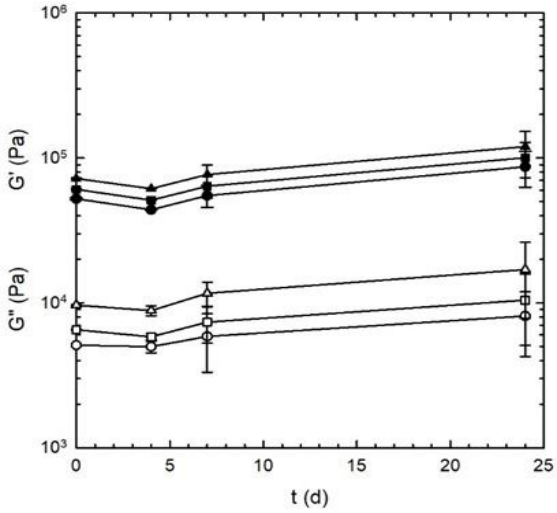


Figure 6: Variation of G' (filled symbols) and G" (void symbols) with fermentation time at 25 °C, 0.1 % strain, and 0.631 (circles), 6.31 (squares), 63.1 rad/s (triangles up) for fermentation of maize dough inoculated with *L. plantarum* A6. Data are the average of two fermentations.

Conclusion

The combination of rheological determinations with pH, moisture content, starch, reducing sugars measurements, and microbial growth monitoring allowed us to precisely know the changes that occur during pozol dough fermentation as well as the time scale over which such changes are most significant. However, each microorganism has specific behaviors with differences neatly distinguishable. Fermentation is very complex and chemical changes can be observed mainly because in the inoculated doughs modifications are regular and repeatable to some extent between at least two fermentations. The first five days of this process are crucial since the most significant changes in composition occur in this period, despite the differences in the reproduction of *Sii* 25124 and *L. plantarum* A6. In this way, it seems unnecessary to extend fermentation beyond one week. On the other hand, the viscoelastic behavior of the dough remains practically unchanged regardless of the inoculated strain and fermentation time. This means that the macroscopic behavior of the dough is governed by the solid-to-liquid ratio and changes in residual starch, being microscopic in nature, are not enough for viscoelasticity to be significantly modified.

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