Active proteases in pozol fermentation

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Introduction. Pozol is a fermented maize dough beverage that is consumed by various ethnic groups in the southeastern of Mexico. A wide variety of microorganisms have already been isolated from this spontaneous fermentation; these microorganisms include fungi, yeasts, lactic acid bacteria, and non-lactic acid bacteria.
It has been reported the presence of proteolytic bacteria since the first 4 to 8 hours of fermentation, reaching its maximum at 30 hours (approximately 10^8 ufc/g) [1]. Whereas little is known about the nature of these proteases and if they are active during the fermentation, the aim of this study is to identify the active enzymes in pozol *in situ*.

Methods. Pozol fermentation was followed for 2 weeks, samples were taken at 0, 1, 3 and 15 days and the pH was measured. Two grams of each fermentation time was used for the soluble protein extraction following 1st, the protocol previously standardized in the laboratory [2] and 2nd, by trying the addition of an alpha-amylase before the extraction procedure. Reducing sugars were determined by the method of Miller. Samples were precipitated with ethanol and suspended in the charge buffer for the SDS-PAGE gel electrophoresis. Proteins were separated according to the method described by Laemmli [3]. For the zymogram, casein was incorporated into the separating gel.

Results. As expected, the pH during fermentation of pozol decreases from 7.69 to 4.68, and remained constant until the 15t^h day, confirming the previously observed results concerning the rapid growth of lactic acid bacteria at the beginning of the fermentation (Fig. 1).

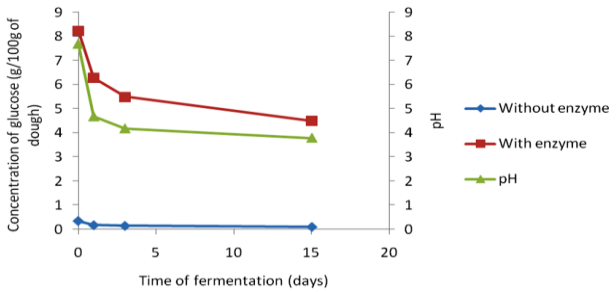

Fig.1 Concentration of reducing sugars and pH in a sample of pozol to different fermentation times.

Whereas starch can form networks that entrap proteins, we decided to incorporate an enzyme in the process of extraction; after testing, work enzyme concentration were established in 12U of alpha amylase /g fresh pozol. The activity of the enzyme in the dough was verified by the release of reducing sugar (Fig. 1).
The extracted protein was analyzed in zymograms for protease activity. Figures 2 and 3 show the obtained results.

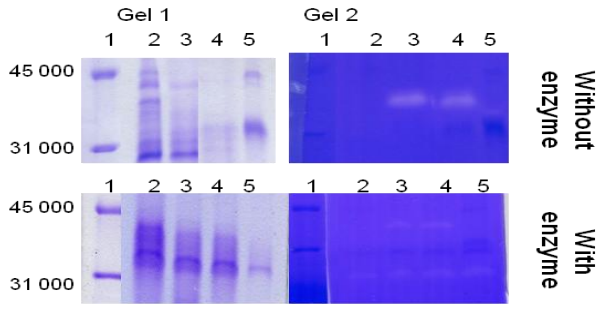

Fig.2 . A: **Gel1**: SDS-PAGE. **Gel 2**: Zymogram with 0.05% of casein. 1) Molecular weight marker, 2) Non-fermented pozol (T0), 3) Pozol fermented for 1 d, 4) Pozol fermented for 3 d and 5) Pozol fermented for 15 d.

In samples obtained without the use of amylase a single protease activity band, with a molecular weight of approximately 42 KDa, was observed in days 2 and 3 of fermentation, but when the amylase is included in the extraction, an additional activity band, with a molecular weight of approximately 21 KDa, can be observed in all fermentation times, including zero, at which point there is still no bacterial growth. These data suggest that the activity originates from the substrate, ie the maize itself. Both proteases will be sequenced for subsequent analysis

Conclusions. It´s possible identify the active proteases involved during the fermentation of pozol *in situ*. As anticipated, the use of the enzyme allows the identification of another protease activity band.

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