

# Changes in Nixtamalized Corn Flour Dependent on Postcooking Steeping Time

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## ABSTRACT

Cereal Chem. 79(1):162–166

We studied the effect of steeping time on various physical and chemical properties of maize flour prepared by the traditional nixtamalization process as well as in oversaturated calcium ion conditions. The calcium content of the corn flour was measured by atomic absorption spectroscopy and was correlated with X-ray diffraction, viscosity, and pH measurements. Calcium content of the flour showed a nonlinear dependence on steeping time, with a local calcium maximum occurring at  $\approx 7$  hr.

The pH level of the corn flour increased with steeping time, thus roughly following the time trend shown by the steeping time dependence of the calcium content. Flour crystallinity and peak viscosity of water suspensions of the flour reached maximal values at  $\approx 7$ –9 hr of steeping, in agreement with manufacturing experience showing that these are appropriate steeping times to prepare tortillas with the desirable rheological and organoleptic properties.

Alkaline cooking of maize to produce dried masa flour, tortillas, and snack foods such as maize and tortilla chips is becoming more important as the popularity of Mexican foods increases in the world. During the last three decades different techniques have been developed to optimize the nixtamalization process (Molina et al 1977; Trejo-Gonzalez et al 1982; Bedolla et al 1983; Johnson and Williams 1992). The traditional nixtamalization process continues to be highly relevant for industrial processing to preserve the quality of the final product. The influence of alkaline cooking on the thermal, rheological, and structural properties of maize flour has not been established. According to Bryant and Hamaker (1997), a better understanding of the interaction between starch (amylose and amylopectin) and calcium hydroxide may be the key to a better understanding of the formation of masa (flour) through the alkaline cooking process. During the nixtamalization process, partial gelatinization of the starch produces important changes in the thermal, structural, and rheological properties of the tortillas. These changes depend on the calcium concentration (Rodríguez et al 1995; Yañez-Limon et al 1995). The importance of  $\text{Ca}(\text{OH})_2$  in the alkaline cooking process of maize has been studied by several authors (Martínez-Bustos et al 1998; Gomez et al 1989; Paredes-Lopez and Saharopulos-Paredes 1983). Still, the role played by calcium in this process remains ambiguous.

The structural, rheological, and chemical changes in the grain during alkaline cooking affect the functional properties of texture, color, flavor, and shelf life (Gomez et al 1989). Some important aspects of alkaline cooking and steeping of the maize grain are well known. The alkaline solution degrades and solubilizes the components of the cell wall, resulting in the removal of pericarp and a softening of the endosperm structure (Trejo-Gonzalez et al 1982; Paredes-Lopez and Saharopulos-Paredes 1983) and allowing a diffusion of water and calcium ions into the starch granules located in the interior of the grain (Gomez et al 1989).

In the traditional nixtamalization process, after alkaline cooking, the whole maize kernels are steeped (in some cases for up to 24 hr) and washed at least twice to remove the remains of the pericarp

and excess calcium (Rooney and Serna-Saldivar 1987). After this process, the nixtamalized product is ready to produce masa, which can be used to make tortillas, tortilla chips (McDonough et al 1987), and extruded food such as ready-to-eat cereals and snacks (Martínez-Bustos et al 1998).

We studied the calcium diffusion process in the maize kernel as a function of the steeping time after cooking, and its effects on the physical and chemical properties of the instant maize flours. During steeping (0–24 hr), diffusion of calcium ions occurs through the entire grain, and this diffusion depends on the concentration of calcium (Rodríguez et al 1995). For this reason, we studied two different processes. The first one is the traditional nixtamalization process (TNP) under normal saturated conditions; the second one is the nixtamalization process in oversaturated conditions. For a better understanding of this process in terms of the diffusion of water and calcium ions, the maize kernel is considered as a multiple-layer system formed by the pericarp and endosperm.

## MATERIALS AND METHODS

### Sample Preparation

Commercially available Toluca maize was obtained from the local market in Mexico City, and food-grade calcium hydroxide (reagent powder) was obtained from Merck. The initial cooking step of the nixtamalization process used 10 kg of whole maize kernels in 20 L of water. The water contained calcium hydroxide at 2% of the weight of the maize. This lime concentration (normal saturation) is in the range reported previously by Trejo-Gonzalez et al (1982) and Khan et al (1982). The maize kernels were cooked for 1 hr and 40 min at 72°C. After cooking, the maize was steeped for 0, 1, 2, 3, 5, 7, 9, 11, 13, and 24 hr, respectively. Two processes were used. In the first, known as the traditional nixtamalization process, the cooked product was divided into 10 separate containers, each containing 1 kg of maize, 2 L of water, and 20 g of calcium hydroxide; these samples were left to steep separately. After the indicated steeping time, the samples were washed twice briefly with distilled water, dried, and ground into flour. In the second process, which we refer to as the oversaturated process, the entire corn sample (10 kg) remained in the saturated calcium hydroxide solution (20 L of water, 20 g of calcium hydroxide) in one single container, and 1-kg samples of drained corn were removed at the times indicated (producing oversaturated conditions in the sense that, during the course of the experiment, the ratio of calcium hydroxide to the remaining steeping corn increased). Once removed, these 1-kg samples were washed twice briefly with distilled water, dried, and ground into flour. In each of the two processes, the drained and washed kernels were dried in an air oven at 40°C, milled in a hammer mill, sieved through a U.S. no. 60 screen, and flash dried to 10% humidity to obtain instant corn flour.

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## X-ray Diffraction

The analysis was done using the method of Rodriguez et al (1996). The nixtamalized product was ground into a fine powder and passed through a 150- $\mu\text{m}$  screen. The samples in powder form were then densely packed into an Al frame. The X-ray diffraction patterns of the samples were recorded on a diffractometer (Siemens D5000) operating at 35 kV and 15 mA, with a Cu  $K_{\alpha}$  radiation wavelength of  $\lambda = 1.5406 \text{ \AA}$ . Data was collected from  $4^{\circ}$  to  $30^{\circ}$  on a  $2\theta$  scale with a step size of  $0.05^{\circ}$ . The crystalline percentages were calculated by normalizing the integrated diffracted intensity over the  $2\theta$  ranges measured in relation to the integrated noncoherent intensity. The noncoherent intensity was obtained by subtracting the sharp diffracted peaks from the total diffracted intensity. The same procedure was used for all the samples. The spectrum analysis software (Diffract/AT, Socavin VI.2) was used for three measurements for each sample, and the average values were reported.

## Atomic Absorption Spectroscopy

The corn flour samples were prepared using the dry ashing procedure described in the AOAC method 968.08 (1998). The calcium ion concentration was measured with a double beam atomic absorption spectrometer (Analyst 300 Perkin Elmer) equipped with a deuterium lamp background corrector and hollow cathode lamp. The operating conditions for the apparatus were air (12 psi), acetylene (70 psi), flame 422.7 nm, lamp current 10 mA, and slit width 0.7 nm.

## Relative Viscosity

The relative viscosities of the water suspensions of the corn flour dough were determined using a pasting viscometer (Rapid Visco Analyser [RVA] Newport Scientific Narabeen, NSW, Australia). Dough samples were adjusted to a 14% moisture content, and distilled water was added to keep the total weight of water and sample constant at 28 g. The corn flour suspensions were made from instant corn flour that was dehydrated at room temperature for 9 hr and then finely milled in a coffee mill. Dry base material (4 g) with particle size  $<259 \mu\text{m}$  (U.S. no. 60 screen) were suspended in 24 mL of water. The sample was heated to  $50^{\circ}\text{C}$ , then

heated to  $90^{\circ}\text{C}$  at a heating rate of  $5.6^{\circ}\text{C}/\text{min}$ , and held constant at  $90^{\circ}\text{C}$  for 5 min, and finally cooled to  $50^{\circ}\text{C}$  at a rate of  $5.6^{\circ}\text{C}/\text{min}$ .

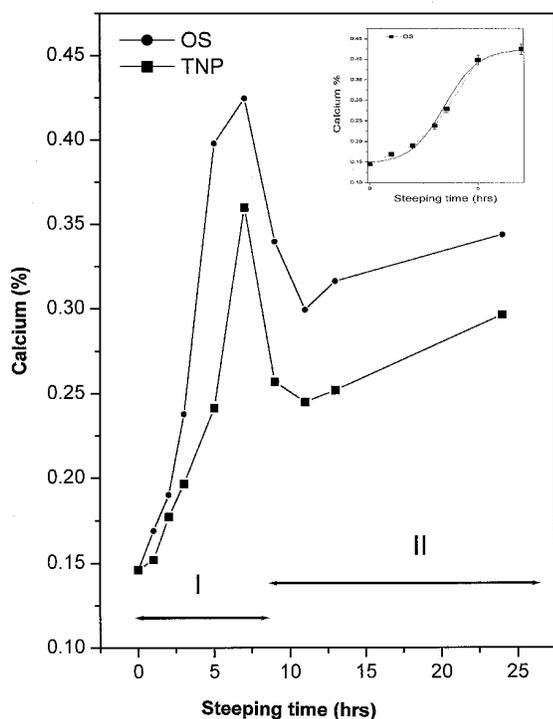
## Sample pH Values

The pH values were determined using a pH meter (Conductronic model pH120) according to Approved Method 44-19 (AACC 2000). The pH meter was calibrated at room temperature with three different buffers (J.T. Baker standard buffers pH 4, pH 7, pH 10). A 10-g sample of corn flour was placed in a beaker containing 100 mL of distilled water and stirred for 15 min to homogenize the sample. The resulting suspension was left to stand for 5 min, and the pH level was read in the supernatant liquid. Readings were taken on three replicate samples and averaged.

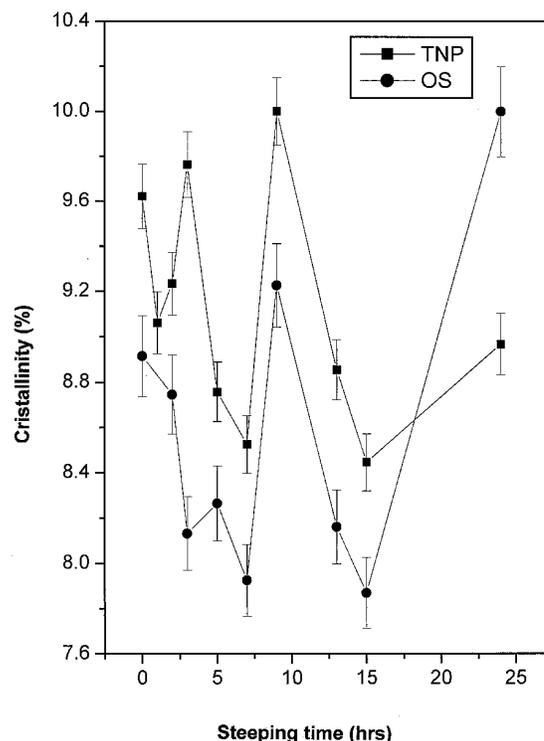
## RESULTS AND DISCUSSION

### Calcium Content

Figure 1 shows the calcium content in the instant maize flour as a function of steeping time for the oversaturated process and for the traditional nixtamalized process. These values represent the average of three measurements per sample. Figure 1 shows that the kinetics of the calcium diffusion through the maize kernel during steeping does not correspond to linear diffusion for either process. Also, in the oversaturated process, we observed that the velocity of this diffusion process is greater than in the normal saturated process. This is due to the greater concentration of calcium ions in the oversaturated solution (Martinez et al 2001). It is interesting to note that for the oversaturated process, the calcium content after 7 hr of steeping is almost three times greater than the calcium content of the corn flour obtained with 0 hr of steeping (i.e., when the kernels were removed right after cooking). The analysis of this process was made using two time schemes. The first time scheme was  $0 < t < 7$  hr; the second time scheme was  $t > 7$  hr. This division is defined by the position of maximum calcium content for both processes. The first time scheme shows a large increase in the calcium content of the corn from 0.15% (w/w, dry basis) at 0 hr of steeping to 0.42



**Fig. 1.** Calcium content of instant corn flour for traditional nixtamalization process (squares) and for oversaturated process (circles) as a function of steeping time. Inset shows best fitting experimental data.



**Fig. 2.** Crystallinity (%) of instant corn flour for traditional nixtamalization process (squares) and for oversaturated process (circles) as a function of steeping time.

and 0.36% for the oversaturated process and the traditional process, respectively, at a steeping time of 7 hr. This increase in calcium content accrues primarily in the pericarp. Experiments by McDonough et al (1987, 2001) showed the calcium in nixtamalized grains were associated largely with the pericarp and the germ. Preliminary results from our study, where the calcium content of the different parts of nixtamalized maize grains was separately assayed by atomic absorption spectrometry, showed the pericarp as the preponderant location for calcium during the early stages of nixtamalization. This process may approach saturation at the later stages of the first time scheme (after  $\approx 3$  hr), indicated by the right curvature of the calcium curve. The sharp decline of the calcium content observed for steeping times  $>7$  hr probably reflects the loss of a large part of the pericarp occurring when these long-treated grains are washed, with the attendant loss of the calcium that had been associated with the pericarp (Trejo-Gonzalez et al 1982). Finally, the gentler rise in calcium content observed for long steeping times ( $>10$  hr) should reflect the slow penetration of the calcium ions into the endosperm. McDonough et al (1987, 2001) reported that calcium generally did not enter the endosperm (or only the outermost layers), but Trejo-Gonzalez et al (1982) did determine that starch obtained from corn nixtamalized using long steeping times had a calcium content almost three times greater than the starch obtained from untreated corn. This implies an entry of the calcium ions into the endosperm, at least for the later stages of the steeping process. Also, preliminary results from our laboratory obtained with nixtamalized kernels from which the pericarp, the germ, and the outermost 10% of the endosperm had been removed, showed that the remaining inner portions of the endosperm had a calcium concentration more than sevenfold higher after 18 hr of steeping, compared with 0 hr of steeping. Other preliminary studies using calcium hydroxide radio-labeled in the calcium ion showed significant entry of calcium ion into the endosperm beginning after several hours of steeping.

The calcium content in the matrix follows an S-shape curve, which typically implies second-order kinetics. This suggests that the calcium diffusion as a function of steeping time can be explained in two time schemes, the first one ( $0 < t < 3$  hr) affecting mainly the peri-

carp, and the second one ( $3 < t < 7$  hr) which increasingly involves the endosperm. S-shape curves (known as logistic curves) (Silva and Miranda 1994) describe second-order kinetics in which the system undergoes a transition between two saturation values taking place in a localized manner. This transition occurs essentially at a given value ( $t_c$ ) during an excursion  $\Delta t$  with a marked change of curvature. Mathematically, the logistic curve can be written as  $f = f_0 + \Delta f [\Theta / (1 + \Theta)]$  where  $\Theta = \exp [(t - t_c) / \Delta t]$ . Here,  $f$  represents the calcium content,  $f_0$  represents the initial value of  $f$  (immediately after cooking),  $\Delta f$  is the overall change of  $f$  in going from the low to the high saturation value; and  $t_c$  is the value of  $t$  at which  $f$  reaches the halfway point of its excursion, changing its curvature in an abrupt manner during a characteristic interval  $\Delta t$  around  $t_c$ . The logistic behavior is typical of structural transitions. For the oversaturated process in the first time scheme ( $0 < t < 7$  hr), fitting the calcium content data to the equation gave values of  $f_0 = 0.146\%$ ,  $\Delta f = 0.214\%$ ,  $t_c = 3.45$  hr,  $\Delta t = 0.779$  hr, and chi-square =  $8 \times 10^{-5}$ . For the traditional process in the first time scheme, the values were  $f_0 = 0.146\%$ ,  $\Delta f = 0.278\%$ ,  $t_c = 3.73$  hr,  $\Delta t = 0.807$  hr, and chi-square =  $8 \times 10^{-5}$ .

### Crystalline Quality

The crystallinity of the nixtamalized corn flour obtained after different steeping times was assessed by X-ray diffraction spectrometry. The results are shown in Fig. 2. A complex pattern qualitatively similar for the two processes (traditional and oversaturated) is observed, in that, starting from a relatively high value of crystallinity at 0 hr of steeping, there is a general decrease in crystallinity to a minimum value occurring at  $\approx 7$  hr, a maximum for 9 hr of steeping, a new minimum at  $\approx 15$  hr, and finally another rise in crystallinity for very long steeping time.

We do not have a full understanding of this complex behavior; however, the following is a possible rationalization. A) During the early part of the steeping process (0–7 hr) the pericarp is the organ most strongly affected, suffering extensive loss in crystallinity (as demonstrated for isolated nixtamalized pericarp by Caballero-Briones et al [2000]), with an attendant loss in the average crystallinity of

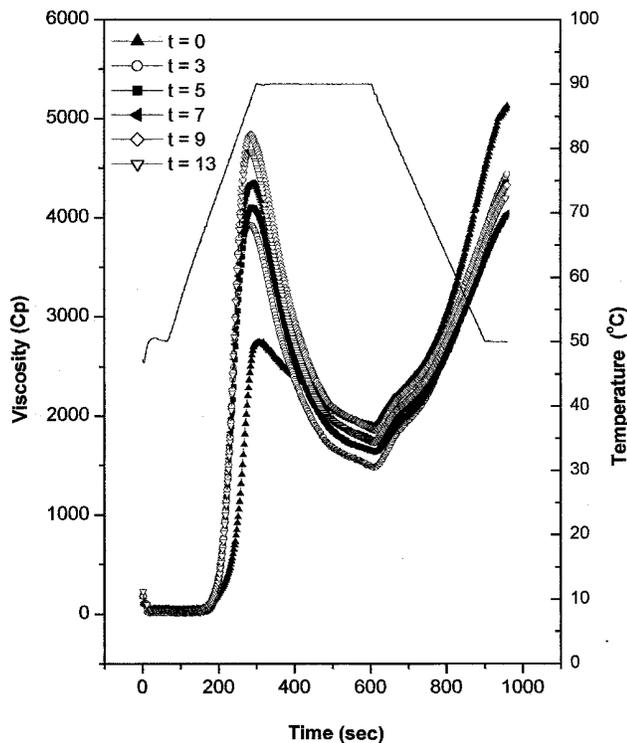


Fig. 3. Amylograms of the instant corn flour for traditional nixtamalization process for samples at  $t = 0, 3, 5, 7, 9,$  and  $13$  hr.

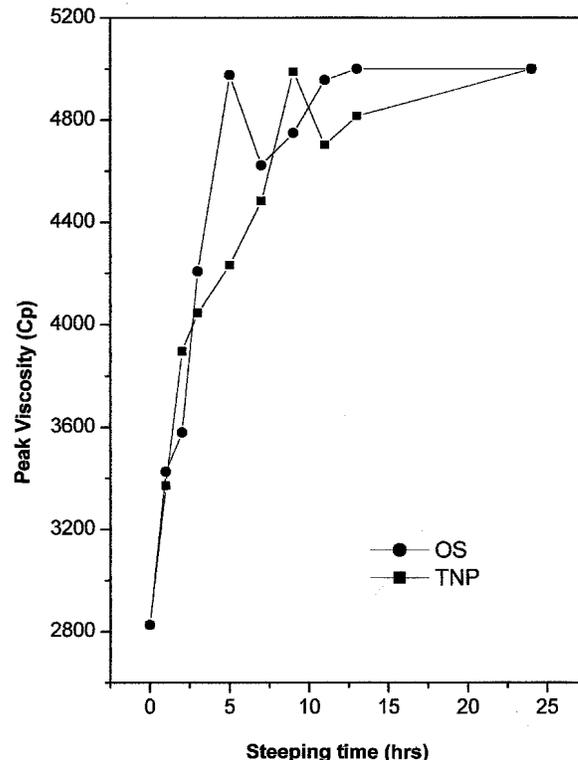


Fig. 4. Peak viscosity for traditional nixtamalization process (squares) and for oversaturated process (circles) as a function of steeping time.

the derived nixtamalized flour. B) In the subsequent stage (7–9 hr), much of the pericarp is lost in the washing of the grains, so that the resulting flour will be relatively enriched in the endosperm fraction, which still retains a large measure of crystallinity. C) The next period shows a decrease in overall crystallinity as the endosperm is being progressively invaded by water and calcium hydroxide. D) The last stage (>15 hr of steeping) shows an increase of crystallinity by slow retrogradation at room temperature.

### Viscosity

Figure 3 shows the viscosity profiles of several instant corn samples. The parameter read from the viscometer curves was the peak viscosity during the heating cycle. Figure 4 shows the peak viscosity as a function of the steeping time for the two experimental processes. The corn flour prepared after 9 hr of steeping for the traditional process showed the highest values of peak viscosity during the heating cycle, while the peak viscosity for the oversaturated process showed a maximum at 5 hr of steeping. The foregoing results are in agreement with the reports by Robles et al (1986). The maximum peak viscosity for the traditional process (TNP) occurs at the same steeping time (9 hr) as the peak for crystalline quality. This could imply, macroscopically, that the calcium ions present in the endosperm after several hours of steeping can help maintain the starch granules intact, and microscopically, that calcium reacts with amylose and amylopectin exuded during the heating phase, producing cross-linking as reported elsewhere (Rodriguez et al 1995; Yañez-Limon et al 1995). That the maximum of peak viscosity occurs at earlier steeping times in the oversaturated process, compared with the traditional process (5 vs. 9 hr), could be due to a more rapid entry of calcium ions under the oversaturated conditions (Fig. 1), or due to the attendant faster entry of hydroxide ions, or due to a faster entry of water favored by a more pronounced weakening of the outermost layers of the kernel under oversaturated conditions.

The viscosity profile can be thought of as a reflection of the granular changes that occur during gelatinization. During the initial heating phase, a rise in maximum viscosity is recorded as granules begin to swell. At this point, polymers with lower molecular weights (amylose) begin to leach from the granules. A viscosity peak is obtained during pasting when there is a majority of fully swollen, intact granules and molecular alignment of any solubilized polymer has not occurred within the shear field of the instrument.

### pH Values

Figure 5 shows the pH values obtained in the oversaturated and traditional nixtamalization processes as a function of steeping time. According to these results, a correlation exists between the calcium content (Fig. 1) and the pH level (Fig. 5) of the corn flour as a function of steeping time. If we compare Figs. 1 and 5 for the traditional nixtamalization process, it is evident that both exhibit the same trend. This means that when the calcium content increases, the pH values also increase. This behavior occurs over the whole range of steeping time. The maximum peaks for calcium content as well as the peaks for pH values are located at the same steeping time (7 hr). Higher pH values are observed in the oversaturated process, due to the excess of calcium hydroxide. Cations form complexes with multidonor molecules containing favorably oriented hydroxyl groups (Hood and O'Shea 1977) and they influence the structure of the starch granules. Alkalinity affects the physical state of the starch granules by inducing swelling and gelatinization. This swelling may unfold the starch molecules and expose the reactive sites. The presence of significant concentrations of calcium ion in the interior of the endosperm during the later stages of the steeping process implies a significant concentration of the counterion (i.e., hydroxide) and, hence, a highly alkaline situation in the endosperm, which could be conducive to partial deprotonation of the sugar moieties of the starch, with subsequent binding of calcium ion in the form of an alkoxide.

If we compare the crystalline quality for the traditional nixtamalization process (Fig. 2) and the pH value (Fig. 5), it is interesting to see that the relative minimum pH value at 9 hr for the traditional nixtamalization process corresponds to the maximum crystalline quality. At this point, after a careful X-ray analysis, no crystalline calcium compounds were found in the instant corn flour. This is the expected result if calcium is bound in the endosperm in the form of a partial alkoxide with random distribution of calcium positions.

According to previous results (Rodriguez et al 1996), the availability of hydroxyl groups in the starch is the main reason for the association of the starch molecules through hydrogen bonds.

Because foods can vary dramatically in pH value, the effect of pH value on starch gelatinization is an important consideration. The viscosity profiles can vary significantly as a function of pH value, not only for native starch but for modified starch as well. In general, pH extremes tend to have a negative effect on viscosity by hydrolyzing bonds and disrupting the molecular integrity of the granule. Although extreme pH value environments can actually help to gelatinize the starch during earlier stages of heating, the starch soon begins to break down (Atwell et al 1988).

## CONCLUSIONS

This study used exclusively Toluca-type grain. This is an important type of maize, used extensively in the central part of Mexico in the preparation of masa for corn tortilla. Our laboratory will examine other important grains in a similar manner. It is highly likely that the results and trends observed in this study will be repeated, in a qualitative manner, with other corn types.

The results of this study suggest that the calcium diffusion process in the maize kernel during steeping after alkaline cooking is a kinetic process governed by different diffusion processes taking place mainly in the pericarp and endosperm. The steeping of the alkaline cooked grain produces changes in the crystalline quality and rheological properties of the corn starch. The optimal steeping time to

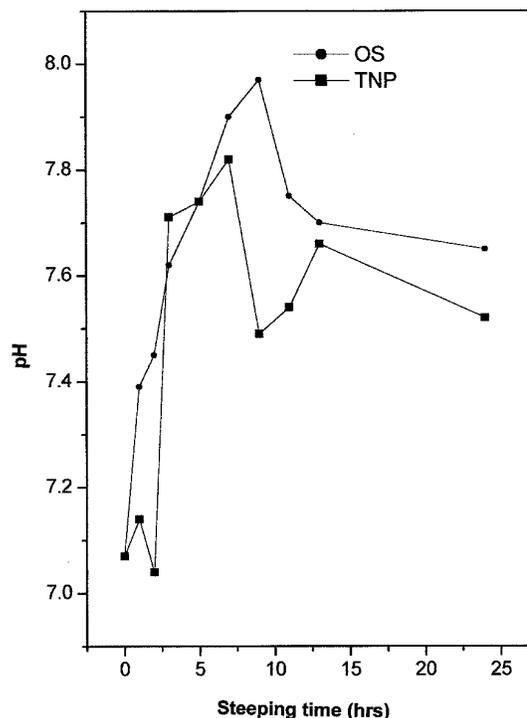


Fig. 5. pH values of aqueous suspensions of instant corn flour for traditional nixtamalization process (squares) and for oversaturated process (circles) as a function of steeping time.

obtain the best crystallinity and rheological properties of instant corn flour for the preparation of tortillas using the traditional nixtamalization process is 7–9 hr. The effect of steeping time on the nixtamalized product is to incorporate calcium ions into the grain in a nonlinear manner. The optimal calcium content for the instant corn flour is  $\approx 0.25\%$  (w/w) (Trejo-Gonzalez et al 1982; Rodriguez et al 1995). This value is reached at 7–9 hr of steeping.

#### ACKNOWLEDGMENTS

This work was partially supported by CONACyT grant 32456-e, COFAA I.P.N., and El Crisol S.A. de C. V., Querétaro, Mexico.

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[Received March 13, 2001. Accepted August 17, 2001.]