A Radioisotopic Study of the Entry of Calcium Ion into the Maize Kernel During Nixtamalization

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ABSTRACT

The entry of calcium ions from the nixtamalization solution into maize kernels over time was followed in model experiments using radiolabeled calcium ions, with autoradiographic evaluation of the kernels after different cooking and steeping times. Calcium ions immediately entered the pericarp and were rapidly fixed at the outer boundary of the endosperm, especially at the external surface of the germ. Entry of calcium into the endosperm occurred gradually after long steeping times, except in the case of broken kernels, for which massive invasion by calcium was observed. After extended steeping times, a moderate amount of calcium-45 was evident in the germ. Specific perforation of the outer layers of the grains provided a defined route of facilitated entry of calcium into the endosperm. No fundamental difference with respect to penetrability by calcium ion was seen in a comparison between flint-type grains and grains containing only floury endosperm.

Corn nixtamalization is the cooking and steeping of maize kernels in an aqueous suspension of calcium hydroxide that is a central step in the conversion of maize to masa and, ultimately, to instant corn flour, snacks, and tortillas. During this treatment, the calcium content of the maize kernels rises considerably, from ≈0.01% (w/w) in the original dry kernel to >0.1% (w/w, dry basis) in the nixtamalized kernels after steeping for 12 hr (Trejo-González et al 1982), an effect which is important from a nutritional point of view, given the importance of calcium in the human diet.

While we have a good idea about the overall rate of entry of calcium into the maize kernel during nixtamalization (Trejo-González et al 1982; Fernández-Muñoz et al 2002), little work has been done to elucidate the penetration routes of the calcium ion and its distribution in various parts of the kernel at different stages of the nixtamalization process. Only Lloyd Rooney's group (Texas A&M University) has addressed this area, exploring the distribution of calcium in the maize kernel after different nixtamalization times (McDonough et al 1987, 2001). They concluded that, for the early times, calcium ion was largely deposited in the pericarp and in the germ, with some penetration up to and into the aleuronal layer, and perhaps even into the outermost layers of the endosperm.

The technique used by Lloyd Rooney's group to determine the location of the calcium ions was thin sectioning of the nixtamalized kernels followed by treatment with a calcium-specific alizarin stain, which produced red crystals visible under light microscopy. A drawback of this type of analysis is that it involves a prolonged process during which the metal ion, if only weakly held in its environment, might conceivably be leached out of its location during the detection process. Another more significant limitation is that this method does not distinguish between the endogenous metal ion and the ion that entered the grain from the calcium hydroxide solution during the nixtamalization process.

The availability of a radioactive calcium isotope of suitable characteristics allows analyzing the entry of calcium ion into the maize kernel under nixtamalization conditions using the method of autoradiography on slices of nixtamalized grains. Calcium-45 is an isotope with a relatively long half-life (165 days), which allows high-quality autoradiograms to be obtained even with the use of relatively low specific radioactivity, and it is a β-emitter of intermediate energy (0.255 MeV), conducive to finely detailed images upon autoradiography. This method of analysis permits the rapid and direct examination of a large number of kernels in reasonable detail, and it specifically displays the distribution of the new calcium entering the grain during the nixtamalization process because the endogenous calcium is not radioactively labeled and, hence, remains invisible with this method of visualization.

MATERIALS AND METHODS

The maize grains used were either a flinty hybrid cultivar of white dented maize from Celaya, Mexico, with a small kernel containing both corneous and floury endosperm, used in the industrial production of masa, or *pozolero tapatío*, a large-grain maize containing only floury endosperm. The kernels used in the experiments were selected based on apparent integrity on visual inspection, as well as for relatively uniform kernel weight for a given experiment. In one experiment, a specific perforation of the outer layers of the grains was made on the flat face opposite the side of the embryo, using a fine drill to form a conical hole ≈0.5 mm deep and 1.5 mm in diameter at the base of the cone.

Calcium hydroxide solutions of 0.12% (w/v) concentration (0.016*M* $Ca(OH)_2$) were prepared by dissolving, at 20°C, the calculated amount of reagent-grade calcium hydroxide (J.T. Baker) in freshly vacuum-degassed deionized distilled water. At the beginning of a nixtamalization experiment, the calculated amount of $^{45}CaCl_2$ (1.0 mCi/mL, 1.0 m*M* $CaCl_2$, Amersham) was admixed at 20°C to the calcium hydroxide solution to achieve a final concentration of 0.5 μCi/mL (0.5 μ*M* $CaCl_2$). In these solutions, the molar ratio of hydroxide ion to chloride ion was 32,000:1.

The nixtamalization experiments were conducted in polypropylene bottles with screwcap closures, heated in a temperature-controlled water bath. The radiolabeled calcium hydroxide solution was preheated to 85°C and the maize kernels were added at a ratio of 1 g of grains to 9 mL of calcium hydroxide solution. The mixture contained in the closed bottle was heated for 75 min at 85°C in the waterbath. Subsequently, the temperature of the waterbath was gradually lowered to room temperature over several hours. Similar steeping temperature profiles were used in all experiments. A typical example is shown in Fig. 1. At different times, the bottle was opened to take out samples of the solution for estimation of its pH (using Universalindikator strips, pH 0–14, from Merck), for liquid scintillation counting, or to take out maize kernels for examination. These grains were gently dabbed dry with tissue paper, visually examined for damage, washed for 5 sec in distilled water, dabbed dry again, carefully freed of their pericarp and tipcap, washed again for 5 sec in distilled water, dried again with tissue paper, and finally cut with a razor blade

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into slices ≈2 mm thick. These slices were embedded in a layer of plasticine, in a regular array, leaving their cut surface exposed. Finally, the entire array was autoradiographed for 48 hr at 4°C using Kodak Bio Max film and automatic film development.

In many cases, the autoradiographic images of the kernel slices were surrounded by a halo due to incomplete closure of the plasticine around the kernel sample, allowing the usually strong radioactive signal at the grain surface to radiate outward. In these cases, xerographic images of the kernel slices were superimposed on the autoradiograms, to delineate the outside limit of the kernel, which was drawn as a white contour in the autoradiogram. Autoradiographic signal outside this contour is disregarded in the interpretation of the images.

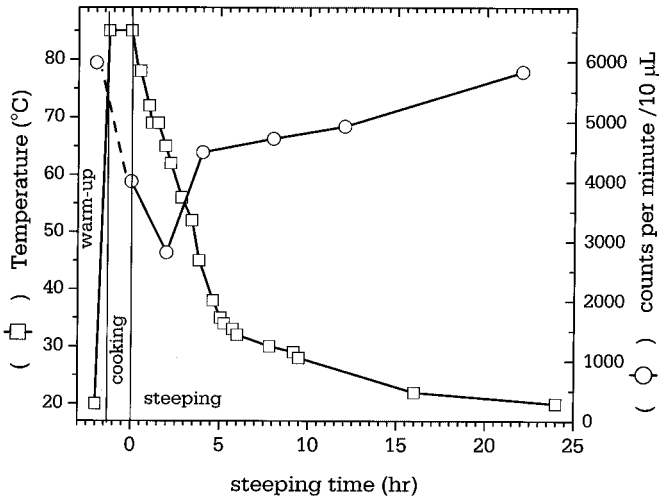

Fig. 1. Time dependence of temperature and radioactivity of the nixtamalization solution for a typical experiment. Temperature of the constant temperature bath (□) and radioactive concentration of the supernatant liquid in the nixtamalization mixture in counts per min/10 µL (○) as a function of time from the original preparation of the radioactive $Ca(OH)_2$ solution at 20°C through heating of the solution and cooking and steeping of the maize kernels.

RESULTS AND DISCUSSION

Due to the special requirements of our experiments with radioactive tracer, the calcium hydroxide solution used in this study differed in composition from the nixtamalization solutions commonly used. The latter typically have a water-to-grain ratio of 2:1 (v/w) and a calcium hydroxide content of 2% (w/w) with respect to grain; that is, 1% (w/v) with respect to water or 0.135*M* aqueous $Ca(OH)_2$. This concentration far exceeds the limit of solubility of calcium hydroxide in water (0.185%, w/w, at 0°C; 0.080%, w/w, at 100°C). Therefore, nixtamalization performed under these conditions proceeds in the presence of copious amounts of undissolved excess calcium hydroxide. We preferred to work at the limit of solubility of $Ca(OH)_2$ (0.12%, w/w, at 20°C), at least at the beginning of the experiment, when radioactive calcium chloride was added at room temperature to the calcium hydroxide solution so as to have an homogeneous solution in which full equilibration and interchange between unlabeled $Ca(OH)_2$ and labeled $CaCl_2$ would be assured. Following the radioactivity of the supernatant solution through time and temperature in one of our experiments, we observed the changes to be expected from considerations of solubility of calcium hydroxide at different temperatures: a reduction in the radioactive counts in the supernatant as the temperature increases to 85°C, and a gradual recovery of the radioactive content of the supernatant as the temperature decreases during the steeping process, indicative of the partial precipitation of calcium hydroxide at the high temperatures and of its gradual redissolution at lower temperatures (Fig. 1). Thus, the radioactive label behaves as expected for regular calcium hydroxide at the concentrations employed.

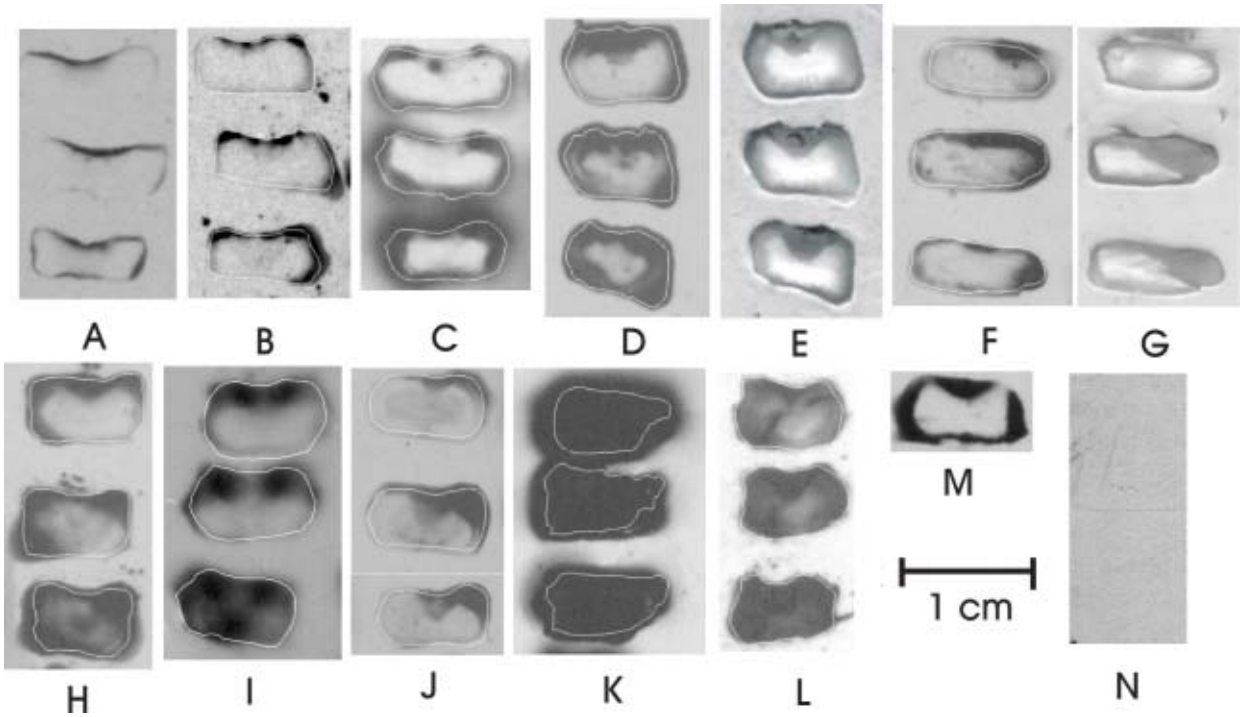

Fig. 2. Autoradiography of slices of maize kernels nixtamalized. in $^{45}Ca(OH)_2$. Kernels cooked at 85°C for 30 min (A) and 75 min (B), and subsequently steeped for 2 hr (C), 4 hr (D, E, F, G), 8 hr (H), 10 hr (I), and 24 hr (J, K, L). E and G are photographs, all other images are autoradiograms. F, G, and K are sequential longitudinal (sagittal) cuts, placed in parallel, with the ventral aspect (the embryo side) topside and the tipcap end on the right. The other images show sequential cross sections, placed in parallel, starting from the distal end of the kernel (top slice of the series) and proceeding to the tipcap end (bottom slice of the series), with the ventral aspect topside. (M) cross section of a kernel dipped in the $^{45}Ca(OH)_2$ solution for 5 sec at 85°C and washed but not peeled. (N) kernel nixtamalized in nonradioactive $Ca(OH)_2$ solution. The white contour lines show the outside edges of the grain slices. Maize cultivar is a flint-type hybrid. Dry kernel weight is 350–380 mg.

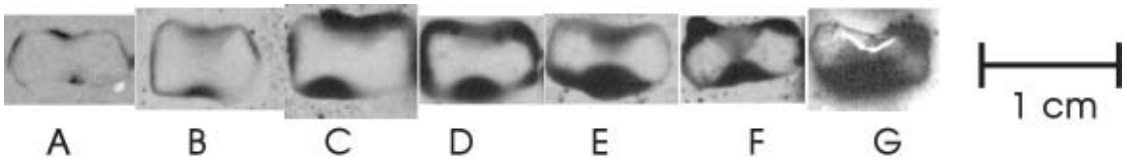

Fig. 3. Autoradiography of preperforated maize kernels nixtamalized in $^{45}Ca(OH)_2$. Kernels cooked at 85°C for 10 min (A), 30 min (B), and 75 min (C), and subsequently steeped for 1 hr (D), 2 hr (E), 4 hr (F), and 22 hr (G). Cross sections were selected to contain the site of original perforation (bottomside). Ventral aspect is topside. Maize cultivar is a flint-type hybrid. Dry kernel weight is 350–380 mg.

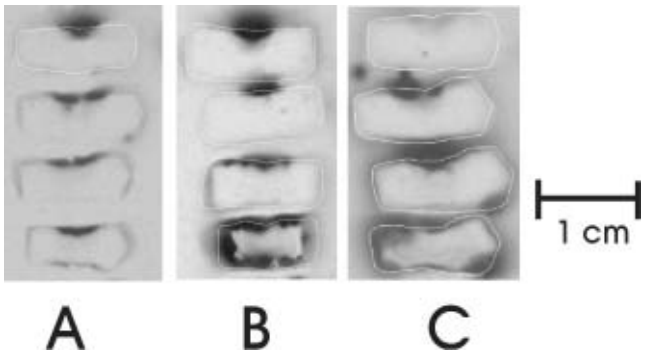

Fig. 4. Autoradiography of kernels of *pozolero tapatío* maize cultivar nixtamalized in $^{45}Ca(OH)_2$. Kernels cooked at 85°C for 75 min (A) and subsequently steeped for 4 hr (B), and 8 hr (C). Cross sections are placed in parallel, starting from the distal end of the kernel (top slice of each series). Ventral aspect is topside. Dry kernel weight is 630–700 mg.

In our experiments, the maize kernels encounter the same concentration of calcium hydroxide as they do in normal nixtamalization because the $Ca(OH)_2$ concentration is saturating, or very nearly so. Still, there remained one concern: under our experimental conditions there would be no ample amount of undissolved calcium hydroxide providing a reserve to assure a constant level of basicity throughout the process. It is for this reason that we worked with a larger ratio of aqueous solution to grain (9:1, v/w) than normally used in nixtamalization (2:1, v/w). With this, our conditions provided for an initial ratio of total calcium hydroxide to dry maize kernels of 0.011:1 (w/w), compared to a ratio of 0.020:1 (w/w) commonly employed in nixtamalization. In an experiment performed without radioactive label, we confirmed that during nixtamalization performed under our conditions the pH of the supernatant solution remained at or above pH 12 (measured at 25°C) throughout the process (data not shown). Also, during the actual experiments with radioactive $Ca(OH)_2$, the solution pH was repeatedly tested with pH strips to ascertain that it had not fallen below 12.

Figure 2 shows the results obtained in a nixtamalization of the flint-type hybrid maize in radiolabeled $Ca(OH)_2$, covering the time from the original cooking up to steeping for 24 hr. The peeled maize kernels analyzed after 30 min (Fig. 2A) and after 75 min (Fig. 2B) of cooking at 85°C contained some radioactivity at their periphery and more concentrated radioactivity at the outside surface of the germ. Otherwise, no internal radioactive calcium was evident in the endosperm or in the interior of the germ.

Figures 2C and D show autoradiographed cross sections of maize kernels after 2 and 4 hr of steeping, respectively. In comparison to the earlier time points, these samples show a progressive penetration of radioactive calcium from the periphery of the grain toward the center of the endosperm, with a preference for the tipcap end. As yet, only the outer part of the germ shows strong radioactivity, while the internal part of the germ (toward the center) is only lightly labeled. This becomes clear from a comparison with the photographic images (Fig. 2E) of the gel slices autoradiographed in Fig. 2D, where the germ is clearly delineated. Similar results are seen in a series of sagittal sections of a kernel after 4 hr of steeping (Fig. 2F and G). Again, only the outside part of the germ is strongly labeled.

Longer steeping times (8, 10, and 24 hr; see Fig. 2H, I, J, K, and L) result in further penetration of calcium into the kernels, though significant grain-to-grain differences are observed. Thus, a kernel steeped for 24 hr (Fig. 2J) may show less overall radioactivity than a kernel steeped for only 8 hr (Fig. 2H). On the other hand, two of the kernels steeped for 24 hr were heavily invaded by calcium (Fig. 2K and L). In these latter two cases, kernel damage was clearly evident at the time the grain was taken from the nixtamalization solution, indicating that the penetration of the maize kernel in its entirety by calcium ion is strongly inhibited so long as the outer structures of the kernel remain intact.

After long steeping times, radioactive label in the entire germ becomes appreciable, though much stronger labeling can be seen in the heavily invaded portions of the endosperm (Fig. 2J).

In general, the radiolabel on the kernel surface shows a distinct bias for the ventral side (external surface of the germ). Sometimes, however, this signal does not appear in a coherent zone but rather concentrated in the interstitial regions where the interface between germ and endosperm meets the outside surface of the grain, giving rise to two strong radioactive foci in the cross sections. An example of this is seen in Fig. 2I, which corresponds to a kernel steeped for 10 hr.

Figure 2M shows a cross section of a grain that had been dipped for only 5 sec in the hot radiolabeled $Ca(OH)_2$ solution. This kernel was washed using the regular procedure but was not peeled. The autoradiogram shows a very intense signal at the periphery, indicating that a high amount of calcium from the nixtamalization solution becomes fixed in the pericarp almost instantaneously.

A control experiment in which kernels were treated similarly in an unlabeled $Ca(OH)_2$ solution with 2 hr of steeping gave no signal on the autoradiogram (Fig. 2N), indicating that the film response was specific for the radiolabel.

Several other similar experiments (data not shown) confirmed the general trends delineated in Fig. 2: immediate fixation of radioactive calcium in the pericarp, early penetration of calcium up to the aleuronal layer, preferential association of the calcium with the outside surface of the germ and with the interstices between germ and endosperm, late entry into the endosperm with a preference for the tipcap end, late entry into the germ, and massive penetration of the endosperm in the case of damaged kernels.

The importance of surface lesions for the entry of calcium ions into the grain was examined in a more controlled manner in another experiment, using otherwise intact kernels that had been perforated on the flat face opposite the embryo side to a depth of ≈0.5 mm. Nixtamalization and analysis of these kernels as in the preceeding experiments showed virtually no entry of calcium-45 after cooking for 10 min at 85°C (Fig. 3A) but a limited extent of penetration at the site of the perforation after cooking for 30 min (Fig. 3B). There was strong local penetration after 75 min of cooking (Fig. 3C) and after subsequent steeping for 1 hr (Fig. 3D), 2 hr (Fig. 3E), 4 hr (Fig. 3F), and 22 hr (Fig. 3G). Interestingly, the penetration in depth of the radioactive calcium through the perforation seemed to slow down rapidly after 1–2 hr of steeping, possibly due to the progressive decrease in temperature, which by this time had descended to ≈60°C, the temperature of onset of maize starch gelification (Fisher and Thompson 1997), or to the difficulty of feeding a steadily

expanding invasion front through the same narrow perforation. In all cases, after peeling the kernels we found that the initial drilling had penetrated not only the pericarp but had also perforated the aleuronal layer, creating a narrow opening of ≈0.5 mm diameter.

While all of the experiments described so far were conducted with a flinty maize cultivar, we have also briefly examined a different type of maize kernel called *pozolero tapatío*. Usually, this type of grain is not nixtamalized in Mexico, being generally destined for the preparation of *pozole*, a corn soup widely consumed throughout the country, but we decided to examine it in our tests because of its larger size which might make visual interpretation easier and because it contains exclusively floury endosperm in contrast to the flinty hybrid that has a small amount of floury endosperm surrounded by a layer of corneous endosperm. The nixtamalization experiment conducted with intact grains (without perforation) of the *pozolero tapatío* cultivar showed essentially no entry of calcium-45 into the endosperm after 75 min of cooking at 85°C (Fig. 4A) and only minor penetration by the isotope after 4 and 8 hr of steeping (Fig. 4B and 4C). This indicates that the slow penetration of calcium-45 generally observed in these studies is not due to low penetrability of the corneous endosperm (absent in the pozolero tapatío cultivar). Rather, it appears that the outermost layers of the endosperm, possibly the aleuronal layer itself, constitute the principal barrier to the entry of calcium ion.

Sharp autoradiographic images were obtained in this work even though relatively thick slices of nixtamalized grains were used. The fact that there was no major parallax problem must be due to the short mean free path of the calcium-45 radiation in the corn tissue. In a control experiment, we observed that the autoradiographic signal obtained from a sample of dried down $^{45}Ca(OH)_2$ was reduced by more than half when a polyethylene sheet 35 μm thick was placed between the sample and the film. On this basis, we assume that the calcium-45 radiation does not effectively penetrate farther than a few dozen micrometers in the nixtamalized corn slices. Thus, even though the sections employed were rather thick, the film only gathered signal from a thin outer layer of the sample.

CONCLUSIONS

The calcium-45 based technique used in this work provides a simple and reliable methodology to assess the intragrain distribution specifically of the calcium ions proceeding from the nixtamalization solution. The technique gives a detailed picture of this distribution, grain by grain. This turns out to be important based on the results of this study, which show that the rate of penetration of calcium ion into the interior of the grains varies considerably from grain to grain and can increase abruptly in the case of significant kernel damage. In general, previous studies on the entry of calcium ion into the maize kernel during the nixtamalization were based on the analysis of an ensemble of kernels after different steeping times (by atomic absorption spectrometry) and could, therefore, only provide information about the average calcium content of the kernels.

Our grain-by-grain analysis gives us a clearer notion of the events involved in the entry of calcium from the nixtamalization solution into the maize kernel. 1) Calcium ions from the nixtamalization solution become associated with the pericarp of the kernels almost instantaneously. 2) Early in the process (already during the cooking phase) calcium from the nixtamalization solution becomes fixed at the outside of the endosperm, possibly at the aleuronal layer. Also, calcium ion becomes associated with the external surface of the germ and with the interstices between germ and endosperm. 3) During the cooking and the early stages of the steeping process, little if any calcium from the nixtamalization solution enters the endosperm or the germ. 4) After several hours of steeping, calcium ion enters progressively from the outside toward the center of the endosperm. This entry is more pronounced for the kernel part proximal to the tipcap end. 5) After long steeping times, calcium from the nixtamalization solution also becomes evident in the germ. 6) Massive entry of calcium into the interior of the endosperm occurs only in those grains that have lost their integrity.

The original corn is relatively low in endogenous calcium (Trejo-González et al 1982), hence the interest in the increase in calcium content accrued during nixtamalization. Our present work shows that, for intact maize kernels, newly entered calcium becomes important in the internal parts of the kernels (endosperm and germ) only after long steeping times. The outer structures (pericarp) bind calcium almost immediately. These, however, could be lost during extensive washing of the nixtamalized grain. Therefore, those nixtamalization methods that combine short steeping times with extensive washing procedures could lead to a nixtamal product relatively poor in calcium content.

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LITERATURE CITED

Fernández Muñoz, J. L., Rodríguez, M. E., Pless, R. C., Martínez-Flores, H. E., Leal, M., Martínez, J. L., and Baños, L. 2002. Changes in nixtamalized corn flour in dependence of postcooking steeping time. Cereal Chem. 79:162-166.

Fisher, D. K., and Thompson, D. B. 1997. Retrogradation of maize starch after thermal treatment within and above the gelatinization temperature range. Cereal Chem. 74:344-351.

McDonough, C. M., Tellez-Giron, A., Gomez, M., and Rooney, L. W. 1987. Effect of cooking time and alkali content on the structure of corn and sorghum nixtamal. Cereal Foods World 32:660-661.

McDonough, C. M., Gomez, M. H., Rooney, L. W., and Serna-Saldivar, S. O. 2001. Chapter 4: Alkaline-cooked corn products. In: Snack Foods Processing. E. Lusas and L. Rooney, eds. Technomic Publishing Co.: Lancaster, PA.

Trejo-González, A., Feria-Morales, A., and Wild-Altamirano, C. 1982. The role of lime in the alkaline treatment of corn for tortilla production. Modifications of proteins: Food, nutritional and pharmacological aspects. Pages 245-263 in: Advances in Chemistry. R. E. Feeney and J. R. Whitaker, eds. American Chemical Society: Washington, DC.

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