Study of Structural and Thermal Changes in Endosperm of Quality Protein Maize During Traditional Nixtamalization Process

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ABSTRACT

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This work presents the study of the structural changes of the endosperm of Quality Protein Maize (QPM H-368C), modified by alkaline cooking at two different temperatures (72 and 92°C) and steeping time of 0–7 hr. Structural changes in the outermost 10% layers, the subsequent 10%, and the remaining 80% of the endosperm as a function of the steeping time were studied using scanning electron microscopy (SEM), X-ray diffraction, and differential scanning calorimetry (DSC) techniques. SEM images revealed that soft and hard endosperm have different shapes and packing factors. The X-ray diffraction patterns of the hard and soft endosperm from raw corn suggest that the hard endosperm consists mainly of amylopectin and has a bigger relative crystallinity quality than the soft endosperm. Samples cooked at 72 and 92°C with and without the $Ca(OH)_2$ and steeped for 0, 3, and 7 hr, showed structural changes, X-ray diffraction patterns from the outermost 10% layers and subsequent 10% of the endosperm were completely amorphous. This fact is related to the total or partial gelatinization of the starch. The crystallinity in the internal layers of endosperm (remaining 80%) did not have significant changes after the treatments and exhibited the characteristic patterns of crystalline amylose and amylopectin. DSC measurements in the outermost layers of the endosperm did not exhibit the characteristic endothermic peak of starch (from 64 to 81°C) compared with the raw sample, while the endotherm peak for 80% of the endosperm internal layers appears in all cases (72 and 92°C). According to these results, a new definition of the nixtamalization process can be developed as follows. During the nixtamalization process there is a total gelatinization of the starch granules from the most external layers, and a partial gelatinization of the innermost internal layers of the endosperm.

Nixtamalization is used to produce many staples food such as tortillas, tortilla chips, and snacks, among others. During the alkaline treatment (cooking and steeping) of corn kernel, one of the most important processes is the diffusion of water and calcium ions into the corn kernel. This process produces important physicochemical changes in the anatomical structures of the corn kernel: pericarp, endosperm, and germ, which are reflected in the nutrimental and sensorial properties of the final products (Bressani et al 1990; Serna et al 1990; Rodriguez et al 1996; Sefa-Dedeh et al 2004).

The high consumption of maize, especially in Mexico, Central America, and the southern part of United States, and the well-known lysine and tryptophan deficiencies in maize protein, create the necessity to develop maize with high quality protein, (QPM). The conversion of maize into tortilla or instant corn flours involves a thermo-alkaline process that changes the physicochemical and nutrimental properties of the original maize. According to Bressani et al (1990), the nixtamalization process in QPM increases the Ca content; they found that the protein quality was significantly higher in tortillas than in raw maize.

The nixtamalization process has been studied over the last four decades, but little work has been done in terms of the structural changes that take place in the whole endosperm, especially during the steeping step. The physical and chemical transformations that take place during the nixtamalization process, studied through the structural changes, can be an excellent tool to understand this complex problem. The structural changes in starch are associated with the swelling of starch granules and such processes as gelatinization, melting, pasting, and retrogradation (Dengate 1984).

Zobel et al (1988) explained the application of the term melting to starch gelatinization based on X-ray and synthetic polymer technologies. Diffraction studies show that crystalline specimens yield reflections from crystal planes. After melting, these reflections disappear and a broad halo appears, indicating a change from a crystalline to an amorphous (molten) state. There is widespread interest in the process of starch gelatinization, in which the organized granule structure is disrupted. Atwell et al (1988) defined starch gelatinization as the process that takes place when starch granules are heated in the presence of water, resulting in the disruption of molecular order within the starch granule. This process is manifested by irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization. Atwell et al (1988) described another physical behavior of starch called retrogradation, the process wherein the molecules composing gelatinized starch begin to reassociate, leading to a more ordered structure. Under favorable conditions, the ordered structure may develop into crystalline forms. This phenomenon is related to the stability of a starch paste during storage annealing.

A review of the classification of native starch was published recently by Sajilata et al (2006). This study classified several types of starches designated as A-type, B-type, C-type, and an additional form called V-type. These starches have been identified based on X-ray diffraction patterns. The A-type is characteristic of the cereal starches, the B-type of tuber and high-amylose starches and retrograded starch. The C-type, which is a mixture of A- and B-type diffraction patterns, has also been identified as characteristic of many legume starches. The V-type applies to single helices of amylose cocrystallized with other compounds such as iodine or lipids.

Using X-ray diffraction, Gomez et al (1991) studied the structural changes of nixtamalized corn flours used to prepare tortilla and tortilla chips. They found that during the production of nixta-

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malized corn flours there are few changes in the organization of starch polymers. They also found that the crystallinity of starch in masa was similar to that of raw corn but with a better recrystallization pattern, even though the corn in masa had been alkaline-cooked, steeped, and ground. In this study, evaluation of the particles was made without taking into account their morphological origin. This means that the particles can be located in the external or internal layers of the endosperm. According to Gomez et al (1992), the cooking step disrupted the crystalline structure of the corn starch, and the starch recrystallization and annealing occur during the steeping step. This study was focused on the crystalline changes of the whole endosperm.

During the nixtamalization process, the calcium content of the maize kernels in the corn cultivar Toluca rises from 0.06% in the untreated dry kernel to >0.32% in the nixtamalized kernels after steeping for 24 hr (w/w, db) (Fernandez et al 2002) and from 0.06% in the native QPM corn kernel to 0.21% in nixtamalized corn kernels after 15 hr of steeping time (w/w, db) (Gutierrez et al 2007).

Rodriguez et al (1995, 1996) studied the changes in the physicochemical properties of nixtamalized corn flours and tortillas with calcium concentration between endogenous (0.07%) and 0.5%. For tortillas containing calcium at 0–0.25% (w/w), the thermal diffusivity, peak viscosity, and crystallinity increased when the calcium content increased. This means that the physicochemical properties of these products are dependent on calcium content. The opposite trend was observed for calcium content >0.25%. In corn flours, it was found that the viscosity increases if the calcium content increases to 0.25%; the opposite trend was found for instant corn flour with high calcium content. More recently, Ramos et al (2004) studied three different corn cultivars (Celaya, Toluca, and Palomero). They showed that the rate of hydration is strongly affected by the grain integrity and this fact could influence the physicochemical changes in the different components of the corn kernels. These authors also reported that the water diffusion coefficient has thermal dependence.

Changes in the crystallinity behavior in the endosperm of nixtamalized corn were analyzed using X-ray diffraction by Mondragon et al (2004). They reported a change in the relative crystallinity of starch structure with lime concentration and steeping. They also suggest that, during nixtamalization, amylose-lipid complexes are developed in the starch, which is supported by the X-ray diffraction patterns.

Another widely used method for studying the structural properties of the starch is differential scanning calorimetry (DSC). This technique is particularly well suited for investigating the phase transition of starch and water systems because it allows the study of starch gelatinization over a wide range of starch and water ratios, the determination of gelatinization temperatures at >100°C, and the estimation of transition enthalpies. The order-disorder transitions that occur on heating an aqueous suspension of starch granules have been extensively investigated using DSC (Lelievre and Mitchell 1975; Donovan 1979; Jenkins 1994). Starch transition temperatures and gelatinization enthalpies measured by DSC may be related to characteristics of the starch granule such as degree of crystallinity (Krueger et al 1987). Gelatinization temperatures and enthalpies associated with the gelatinization endotherms vary for different corn starches. Sandhu et al (2004) observed that the transition temperatures of normal corn starches are 64.0–76.8°C.

During nixtamalization, starch undergoes functional and structural changes that depend not only on corn characteristics but also on processing conditions. Analytical methods that evaluate pasting and thermal properties have been used to investigate changes in starch during nixtamalization (Robles et al 1988; Gomez et al 1991; Almeida et al 1997). Sahai et al (1999) reported that DSC can be a useful indicator for determining the degree of cook as well as for evaluating the impact of processing conditions on nixtamalized products. Yglesias et al (2005) investigated the thermal properties of ground raw corn, freeze-dried nixtamal, and masa at different cooking temperatures (86–96°C) and steeping times (3–11.77 hr). Nevertheless, they did not study the relationship between the calcium content and the DSC enthalpy peak. These authors concluded that nixtamal DSC enthalpy was affected mostly by steeping time, while masa enthalpy was affected exclusively by cooking temperature. Krueger et al (1987), Robles et al (1988), and Sahai and Jackson (1999) suggested that endotherm peak temperatures and enthalpy values were greatly influenced by steeping time due to the annealing phenomenon that takes place at near gelatinization temperatures. However, all these studies were made considering corn kernel as an entire system. It is very important to emphasize that the corn kernel consists of different anatomical structures (pericarp, germ, and endosperm) and it is necessary to take into account that the endosperm consists of soft and hard layers with different packing factors and chemical composition. These criteria were not accounted for in previous research.

This work presents a complete study of the structural changes in the endosperm that take place during the traditional nixtamalization process as a function of the steeping time from 0 to 15 hr for samples cooked at 72 and 92°C using scanning electron microscopy, differential scanning calorimetry, and X-ray diffraction. The analysis was done in the 10% of the outermost layers, the subsequent 10%, and the remaining 80% of the endosperm.

MATERIALS AND METHODS

QPM Material

The Quality Protein Maize (QPM) hybrid H-368C was produced by the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP) and Centro de Investigación para el Mejoramiento del Maiz y del Trigo (CIMMyT). The maize was sown on May 2004 at the Bajío Experimental Station (INIFAP) in Celaya, Guanajuato, México. Crop management followed INIFAP recommendations. Ears were hand-harvested in November 2004 and the grain was stored at 4°C until used.

Chemical Composition

Moisture content of corn kernels before and during the cooking step was determined using Method 925.10 and Method 920.86 for crude fiber content (AOAC International 2000). Ash content, protein content, and fat content were measured using Approved Methods 08-01, 46-13, and 30-25 (AACC International 2000), respectively. All the measurements were made in triplicate.

Soft and Hard Endosperm Determination

To determine the amount of soft and hard endosperm, the pericarp and germ were manually removed after soaking corn kernels in distilled water for 3 hr. The kernels were stirred in a 250-mL beaker using a hot plate without temperature. Then the corn was dried at 40°C for 2 hr and finally the soft-to-hard endosperm ratio was calculated. The endosperm, soft and hard, was weighed, and the soft endosperm was carefully removed using a low-speed dental precision hand instrument (COA, International) equipped with a bur diamond.

Corn and Endosperm Hardness

The corn and endosperm hardness was determined in 100 corn kernels using a texture analyzer (model TA-XT2 Texture Technologies Corp, Scarsdale, NY) to determine the breaking force required for different samples of corn kernels. The same test was made on soft and hard endosperm. The area under the force-deformation curve was analyzed with texture analyzer software as reported by Martínez-Bustos et al (1999).

Corn Nixtamalization

For better control of the nixtamalization process for the cooking and steeping steps, as well as reproducibility of the samples, a

nixtamalization computer controlled process (NCCP) was developed to control the cooking temperature and to monitor temperature for the cooking and cooling steps in real time (Gutierrez et al 2007). In the initial cooking step of the nixtamalization process, each sample was prepared by cooking 3 kg of QPM H-368C kernels in a solution of 6 L of distilled water and 30 g of calcium hydroxide (1%, w/w) (reagent powder, Fermont, Monterrey, NL, México). The corn kernels were added to the container and heated to the respective reference temperatures for each treatment. The sample remained in the container at this temperature for a characteristic cooking time that depends on the cooking temperature: 65 min for 72°C and 25 min for 92°C. These temperatures were calculated according to the final moisture content at the end of the cooking step (36%) and the manual removal of the pericarp. After the cooking step, the system was turned off and the temperature gradually decreased. The temperature changes were monitored until the sample reached the chosen steeping time to begin the washing process. Maize kernels were cooked at 72 and 92°C at 65 and 25 min, respectively. After cooking, the maize was steeped for 0–15 hr. The cooking liquor (nejayote) was drained off and the nixtamal samples were washed twice in water (distilled water was used to avoid Ca interference) using a 2:1 (v/w) ratio by stirring the kernels in the wash water for 1 min. After washing, the pericarp, endosperm, and germ of each soaked kernel were separated manually and dried using an air oven at 40°C for 8 hr (each component reached a relative moisture content of 12%). As the endosperm constitutes ≈80% of the whole kernel, the outermost 10% and the subsequent 10% of the dry undamaged endosperm were removed manually, one kernel at a time, by sanding all faces with sandpaper (Fandeli, Sic B-99, 1200) following the procedure reported by Fernandez et al (2004).

Corn Samples Characterization by Low-Vacuum Scanning Electron Microscopy (LV-SEM)

The morphologic analysis of the endosperm in each sample was performed with low-vacuum scanning electron microscopy (LV-SEM, JSM 5600LV) with a resolution of 5 nm in LV mode, fitted with an energy dispersive X-ray spectrometer (Noran instrument, model Voyager 4.2.3). Before analysis, the corn samples were fixed on the specimen holder with carbon tape mounted on an aluminum specimen holder. The analysis was performed using 20 kV electron acceleration voltage and 12–20 Pa pressure in the specimen chamber, obtaining the images on the fracture surfaces with the backscattering electron signal.

X-ray Diffraction Characterization

The X-ray diffraction patterns of amylose (Sigma Chemical A-7046), amylopectin (Fluka BioChemika AG 10120), soft and hard endosperm of QPM nixtamalized endosperm as function of the steeping time, as well as the different layers of the undamaged nixtamalized endosperm (10, 10, and 80%) were collected. The samples were ground into a fine powder and passed through a 150-μm screen. The samples in powder form were then densely packed into an aluminum 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_α radiation wavelength of $\lambda = 1.5406$ Å, and from 4° to 30° on a 2θ scale with a step size of 0.05°. The measurements were performed at room temperature.

Differential Scanning Calorimetry

The first sanding (10%), second sanding (10%), and the 80% remaining of the endosperm (9.1% moisture content) were investigated using DSC (Q100, TA Instruments). A known weight of indium standard was used in the reference pan to balance the heat capacity of the sample pan. Samples (10 mg) were accurately weighed (Ohaus, Voyager) into stainless steel pans (900793.901, TA Instruments) hermetically sealed, hydrated with 55 μL of distilled water and heated from 30 to 110°C at a rate of 5°C/min. All the analyses were performed in a nitrogen atmosphere to 300 kPa (3 atm) internal pressure. Enthalpy of transition (ΔH) was calculated using software for the Universal Analysis 2000 TA Instruments. DSC measurements were made in three replicates and the mean values are reported for all samples.

RESULTS AND DISCUSSION

The chemical proximate analysis of the corn kernels without thermo-alkaline treatment was moisture content 12.1%, ash 1.34, protein 6.66%, lipid 5.12%, and crude fiber content 11.3%. Corn hardness was 14.82 ± 1.34 KgF. In addition, hard and soft endo-

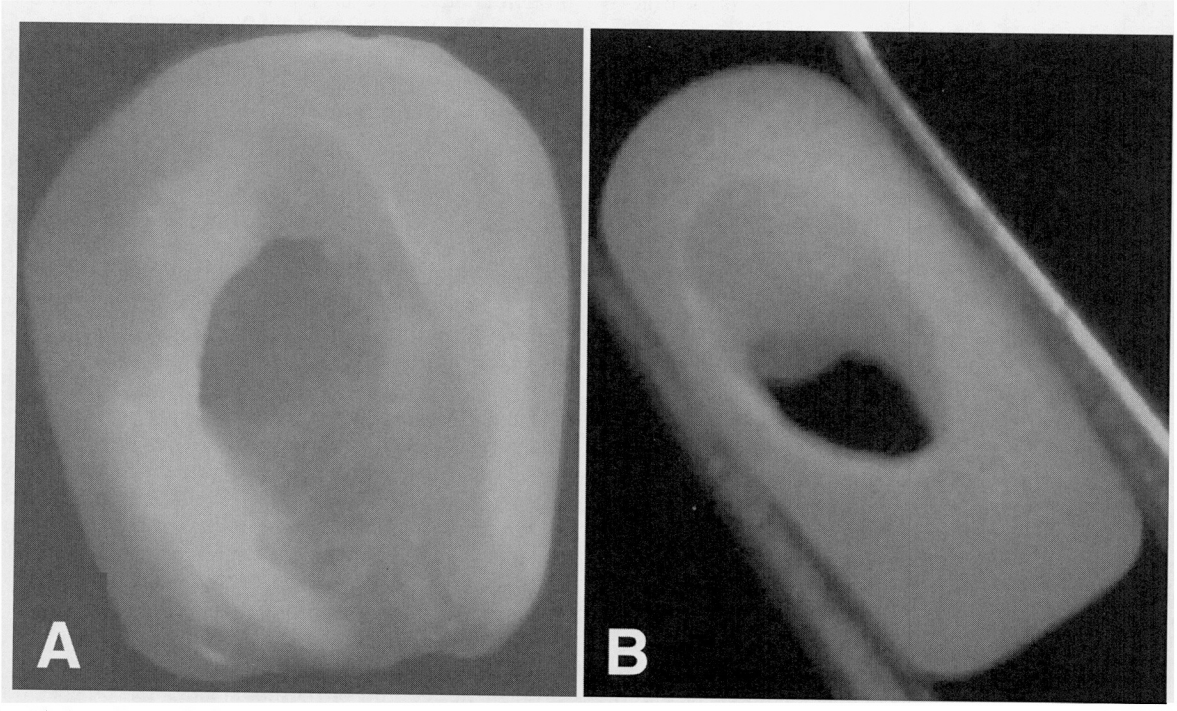

Fig. 1. A, Frontal view of uncooked endosperm of QPM H-368. **B,** Upper view of corn kernel. Holes show location of soft endosperm and germ.

sperm hardness was measured in the same kernels, where values were 12.44 ± 1.11 and 7.99 ± 1.24 KgF, respectively. It is well known that the endosperm content of corn kernels varies from 75 to 85%. The endosperm is mainly formed by starch present as clusters of starch granules covered by a protein matrix in two different packing forms (soft and hard endosperm). The soft and hard endosperm are distributed in the corn kernels in different regions. In QPM H-368, the soft endosperm is located in the top of the corn kernel and behind the germ from the top the tip cap (Fig. 1A and B). Holes in these figures show the location of the soft endosperm that was removed and also the germ. These figures represent total hard endosperm of the uncooked corn kernels. According to the methodology used to obtain the soft and hard endosperm, the soft endosperm constitutes ≈20.82 ± 1.5%, and the remainder is hard endosperm. Usually the ratio of soft to hard endosperm depends on the type of corn. For the dent corn the ratio is 2:1 (Wolf et al 1952).

The soft (floury) and hard (corneous) endosperm ratio is a very important parameter for thermo-alkaline processes because during the cooking and steeping steps, the changes in these structures influence the structural, rheological, textural, and mechanical properties of the nixtamalized products (Rodriguez et al 1995, 1996; Fernandez et al 2002, 2004). SEM images gave a better understanding of the starch changes of the hard and soft endosperm of the uncooked corn kernels. Figure 2A and B show starch granules corresponding to the hard endosperm. Figure 2C and D show the soft endosperm. It is evident that the starch granules of the endosperm are randomly packed; thus, it is not possible to find order in a short range. The packing factor for a random system is defined as the volume fraction occupied by disordered arrays of macroscopic or microscopic particles in contact with one another (Zallen 1998). In the hard endosperm the fraction is bigger than in the soft endosperm. It is possible to see that the shape of soft starch granules is almost spherical, while the hard endosperm exhibits polygonal shapes. The origin of these structures according to Saas et al (1977) is caused by the cell division in the corn kernel. This work also suggests that the less mature cells in the soft (floury) endosperm, located in the central core (Fig. 1), have lower amylose content than cells in the surrounding hard endosperm. These figures are important in the nixtamalization process because the packing factor affects the calcium and water diffusion into the corn kernels.

The endosperm is mainly formed by starch (amylose and amylopectin) and the structural configuration of these polymers can be studied through X-ray diffraction patterns. Figure 3 shows the X-ray diffraction patterns of amylose, amylopectin, and starch of corn without treatment. The amylopectin exhibits the best relative crystallinity quality, while amylose constitutes the less crystalline phase of the starch. The relative crystallinity quality is calculated by normalizing the integrated diffracted intensity over the measured 2θ range to the integrated noncoherent intensity, which is obtained by subtracting the sharp diffracted peaks from the total diffracted intensity (Rodriguez et al 1995, 1996). The relative crystallinity values of amylopectin and amylose were 37 and 22%, respectively. Clearly, the amylopectin macromolecules in the starch granules have a better crystalline order than does amylose.

Figure 4 shows the X-ray diffraction patterns of the hard and soft endosperm from the raw corn (QPM H-368). It is possible to determine that the hard endosperm has a better relative crystallinity quality than the soft endosperm. This could be explained in two ways: the first one relates to the packing factor of starch granules, and the second one refers to the fact that the hard endosperm is mainly amylopectin. Unfortunately there is no accepted defined X-ray diffraction pattern in the International Centre for diffraction

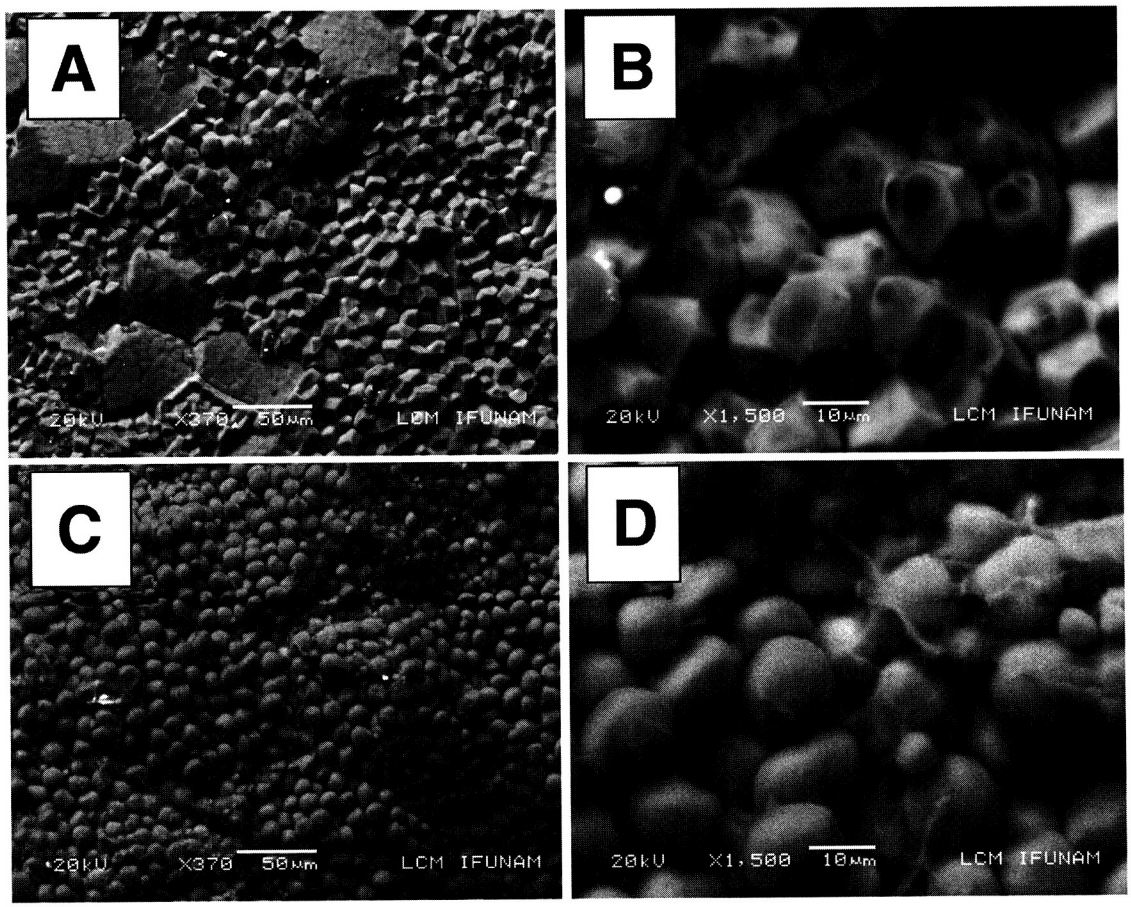

Fig. 2. SEM images of QPM H-368 taken at 350× and 1,500×. **A** and **B,** hard endosperm. **C** and **D,** soft endosperm.

data to compare the X-ray diffraction patterns. By direct inspection of Figs. 3 and 4, it is possible to determine that the hard endosperm could be formed mainly of amylopectin. This is the normal way to identify structures using X-ray diffraction data. According to powder diffraction data for amylose (α-amylose 43–1858) (JCPDS-International Centre for Diffraction Data 1997) (Imberty et al 1988), this structure does not exhibit the peaks located at 15.214 (d = 5.8191 Å), 21.154 (d = 3.8382 Å) in the 2θ scale that are characteristic for the amylopectin identification. These results agree with previous works reported by Gibbon et al (2003) for QPM lines where X-ray diffraction showed that QPM corns are rich in amylopectin.

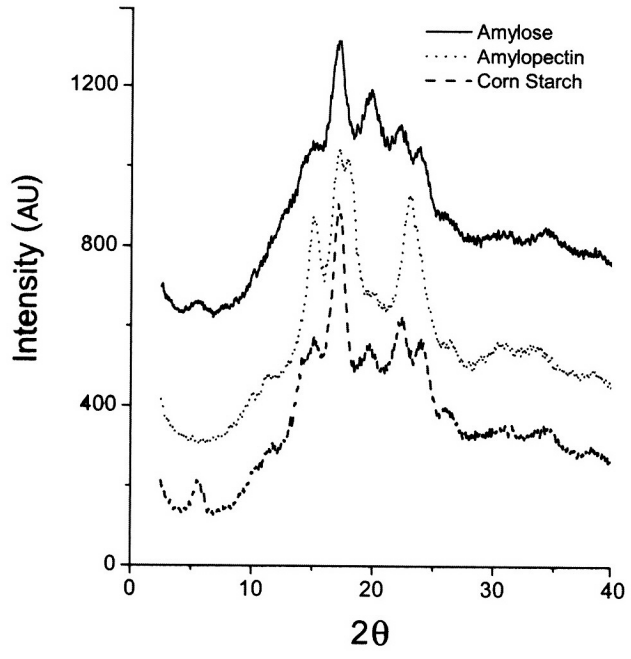

Fig. 3. Characteristic X-ray diffraction patterns of amylose, amylopectin, and corn starch.

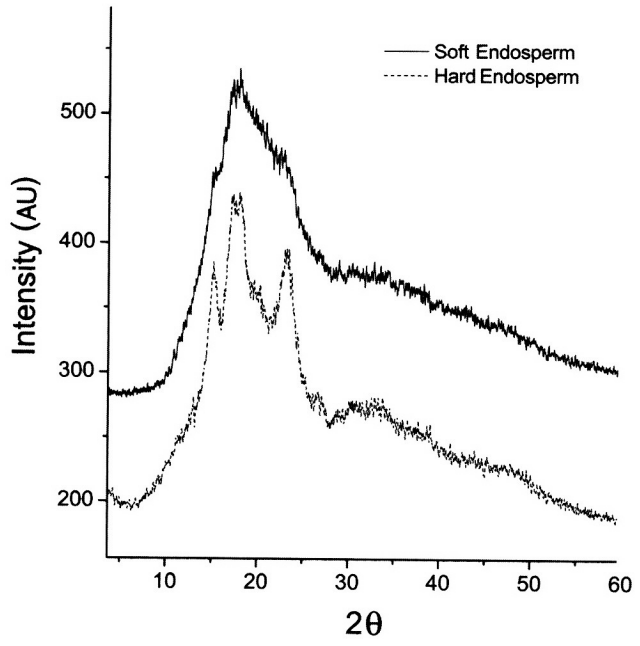

Fig. 4. Characteristic X-ray diffraction patterns of soft and hard endosperm from QPM H-368 without treatment.

As mentioned earlier, this work considers the endosperm as a multilayer system. It is important to study the structural configuration using X-ray diffraction. Figure 5 shows the characteristic X-ray diffraction patterns of corn endosperm without the thermo-alkaline treatment taken as the 10% of the outer layers, subsequent 10%, and the remaining 80%. These patterns correspond to the amylopectin structure (Fig. 3). X-ray diffraction data is a useful and unequivocal tool to structural identification of any crystalline material.

The structural transformation that takes place in the endosperm due to the thermal and thermo-alkaline treatment was studied using DSC and X-ray diffraction techniques. Taking into account that during the cooking and the steeping steps there is a simultaneous water and calcium ion diffusion, it is reasonable to think that the outermost layers of endosperm undergo the initial changes that can be reflected in the structure of the starch granules and on the internal order of amylose and amylopectin.

One of the most important points of this work is the necessity of structural analysis considering the morphology of the endosperm and the fact that the calcium and water diffusion processes take place from the external to the internal layers of the endosperm, producing structural changes as a function of the endosperm position. Fernandez et al (2004) studied the calcium content in the anatomical components of the corn kernels and they were able to determine the calcium content in the pericarp, germ, and endosperm. Their studies were focused into the calcium ion content of the outermost 10%, subsequent 10%, and remaining 80% of this structure. Their results revealed that the calcium is present mainly in the most external layers of the endosperm. However, they did not study the structural changes in anatomical structure.

It is very important to study the structural changes that take place in the endosperm following the changes in the amylose and amylopectin of nixtamalized corn kernels as a function of the steeping time, focusing on the structural changes as function of deep endosperm.

In Fig. 1, the outermost 10% of the endosperm of QPM H-368 maize is hard endosperm, which according to the X-ray diffraction pattern of Fig. 4, exhibits a crystalline phase corresponding to amylopectin (Fig. 3).

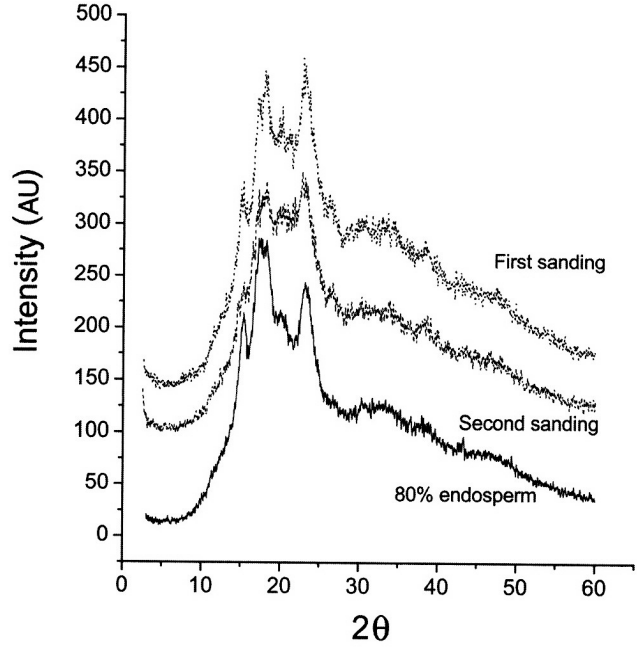

Fig. 5. Representative X-ray diffraction patterns of the 10% outermost layers, subsequent 10% layers and the remaining 80% of the endosperm of corn kernel without any treatment.

Figure 6 shows the X-ray diffraction patterns of the outermost 10%, subsequent 10%, and the remaining 80% of the endosperm after the thermal treatment (72°C). For this purpose, the corn kernel was cooked in water without calcium hydroxide. The X-ray diffraction patterns show that the thermal treatment produces important changes in the most external layers of the endosperm. The starch from this region after this treatment is completely amorphous. On the other hand, the crystallinity in the internal layers of endosperm did not change significantly after the thermal process. For samples cooked up to gelatinization temperature, it is well known that there is total or partial gelatinization of the starch (with appropriate water content) that produces order disruption in the amylose and amylopectin and, as a result, produces loss of the crystalline quality. However, the remaining 80% of the endosperm exhibits a good crystalline quality and a characteristic pattern of amylopectin (Fig. 3). This fact could be related to the low moisture content of the internal endosperm (Almeida et al 1997). The same behavior was found for samples cooked at 92°C.

One important point is the study of the evolution of the crystalline quality of the endosperm as a function of the steeping time for nixtamalized samples. Figure 7A–C shows the X-ray diffraction patterns of the outermost 10%, subsequent 10%, and remaining 80% of the endosperm for nixtamalized samples steeped for 0, 3 and 7 hr at 72°C cooking temperature. The 20% of the external endosperm (hard endosperm) exhibits structural damage reflected in characteristic X-ray amorphous patterns, while in the remaining 80% of the endosperm, the X-ray diffraction patterns exhibit crystallinity as in Fig. 3. It is evident that the amylopectin and amylose content after thermo-alkaline processing in this part of the endosperm change significantly, even at the highest temperature treatment.

Figure 8A shows the X-ray diffraction patterns of the outermost 10% of the layers of the endosperm for samples nixtamalized at 92°C (first sanding) and Fig. 8B shows the second sanding for samples steeped at 0, 3, and 7 hr. In Fig. 8A and B, the outermost layers are amorphous, while in the X-ray diffraction patterns of the internal endosperm (Fig. 8C), the X-ray diffraction patterns exhibit crystallinity. The same experiments were conducted for different steeping times at 0–15 hr and exhibited the same trends.

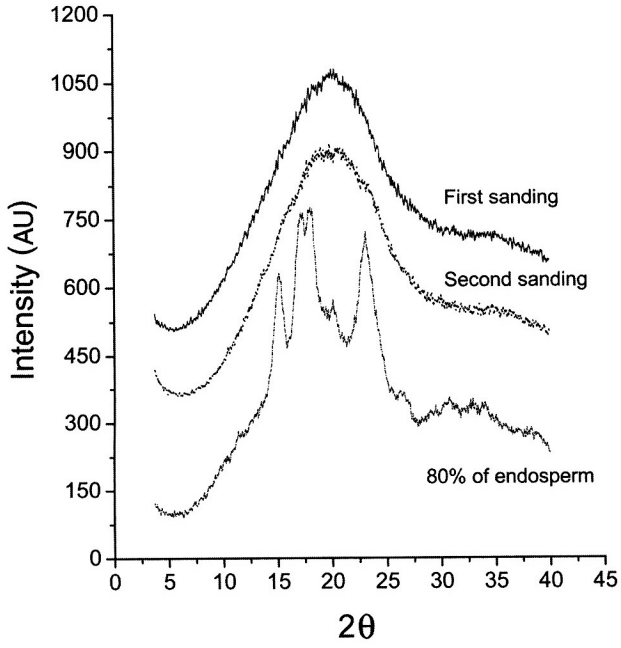

Fig. 6. X-ray diffraction patterns of the 10% outermost layers, subsequent 10% layers, and the remaining 80% of the endosperm of corn kernel with thermal treatment at 72°C.

Crystallization corresponds to the phase in which a system goes from amorphous to ordered system with long or short order. This means that the system has crystalline or polycrystalline phases, while in recrystallization, it occurs when the system recovers or improves crystalline order. In the starch-water system, recrystallization depends on the water content. This process is also time-dependent due to the water bond dependence of the system. According to Figs. 7 and 8, the recrystallization process does not take place in the outermost layers of the nixtamalized endosperm.

Ramos et al (2004) showed less diffusion of water in the internal layers of the endosperm. Almeida et al (1997) reported the relationship between kernel properties and alkaline cooking characteristics of corn with different kernel characteristics. They found that the hard corn endosperm particles consistently required a longer time for hydration and gelatinization of starch. Previous works reported by Gomez et al (1991, 1992) using X-ray diffraction showed that the nixtamalization process produces few changes in the organization of starch polymers. By analyzing the X-ray diffraction patterns of the raw corn, nixtamal, masa, tortilla, and tortilla chips, they concluded that cooking reduces the intensity of the major peaks, indicating that crystalline starch structure was partially disrupted. In the foregoing works, the authors used whole corn kernel for the X-ray diffraction patterns. Changes in some structures with different locations in the endosperm were not detected by this technique. For this reason, the X-ray patterns in these works did not detect the presence of completely amorphous zones from the whole endosperm.

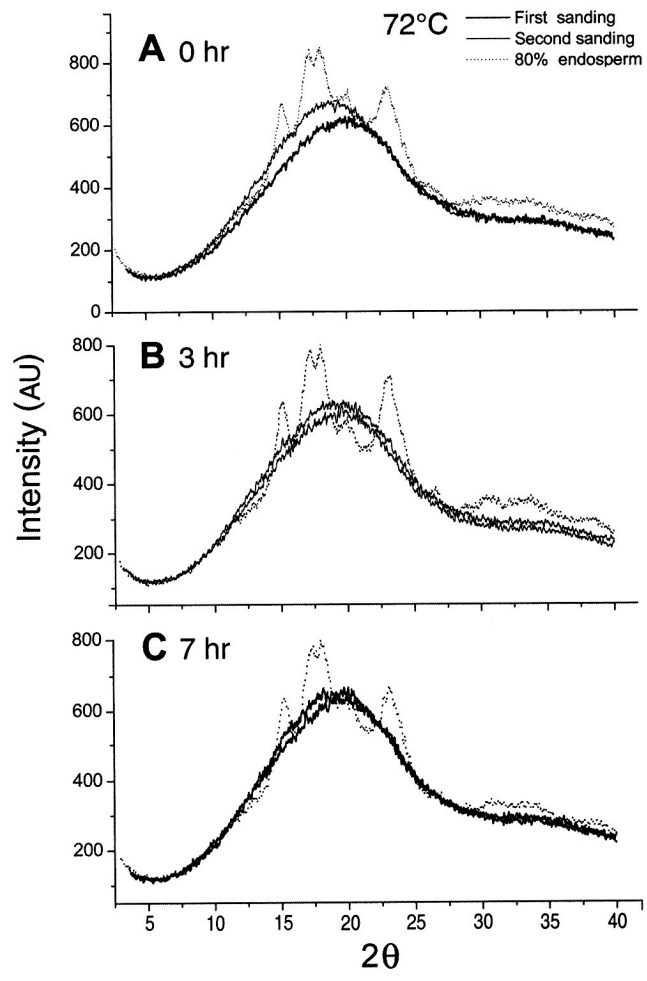

Fig. 7. A, X-ray diffraction patterns of the 10%; **B,** subsequent 10%; and **C,** remaining 80% of the endosperm for samples steeped for 0, 3 and 7 hr at 72°C cooking temperature.

The X-ray diffraction study performed on the endosperm showed that the outermost layers of this structure presented a melting or gelatinization phenomenon due to the thermo-alkaline process, which is reflected in the amorphous patterns (Figs. 6, 7, and 8). To confirm the gelatinization process in the outermost layer of the endosperm, due to the thermal and thermo-alkaline processing, DSC measurements were made in samples cooked at 72 and 92°C.

Figure 9A–C shows the DSC analysis of samples cooked at 72°C and steeped for 0, 3 and 7 hr. Figure 9A shows the DSC of the outermost 10% layers of the endosperm (first sanding). It is clear that the samples from this region of the endosperm do not exhibit the characteristic endothermic peak at 64–81°C (Sandhu et al 2004). The peak for the outermost 10% of the endosperm layers without any treatment under the same conditions was found at 72.91°C, which reflects the melting point of the starch or the gelatinization event. It means that, due to the nixtamalization process, these samples were already gelatinized, as confirmed by X-ray diffraction data shown in Fig. 7A–C. Figure 9B shows the DSC of the subsequent 10% of the endosperm (second sanding); the starch from this region also was gelatinized. Figure 9C shows the DSC of the remaining 80% of the endosperm. It is interesting to see that for 0, 3 and 7 hr of steeping, these samples exhibit the characteristic endothermic peak, which reflects some starch damage.

Figure 10A–C shows the DSC analysis of samples cooked at 92°C and steeped for 0, 3 and 7 hr. By direct comparison with Fig. 9, it is possible to establish that the most internal layers of the endosperm exhibit the characteristic endothermic peak, and by inspection of Fig. 8.C, the origin of this peak could be due to the remaining crystallinity of amylose and amylopectin after thermo-alkaline processing. According to X-ray diffraction patterns shown in Figs. 7 and 8, and the DSC analysis of the outermost layers of endosperm, this structure presented a melting or gelatinization phenomenon due to the thermo-alkaline process.

CONCLUSIONS

According to the results of this study, the QPM H-368 corn kernel consists mainly of hard endosperm, representing 80% of its structure. The soft and hard endosperm exhibit different packing factors and shapes (Fig. 2). The X-ray diffraction patterns showed that hard endosperm could consist mainly of amylopectin. There is no report of an accepted X-ray diffraction pattern for amylopectin so it is not possible to compare patterns, while soft endosperm is mainly formed by amylose according to the X-ray diffraction pattern reported by Imberty et al (1988). The origin of the crystalline structure of the studied corn kernels comes from the amylopectin present in the starch granules (Fig. 3).

The X-ray diffraction patterns of the outermost layers of the endosperm for samples cooked at 72 and 92°C showed that these structures had been disrupted as result of the thermal and thermo-alkaline processing, and that the most internal layers of the endosperm exhibit crystalline phases coming from the crystalline region of amylose and amylopectin.

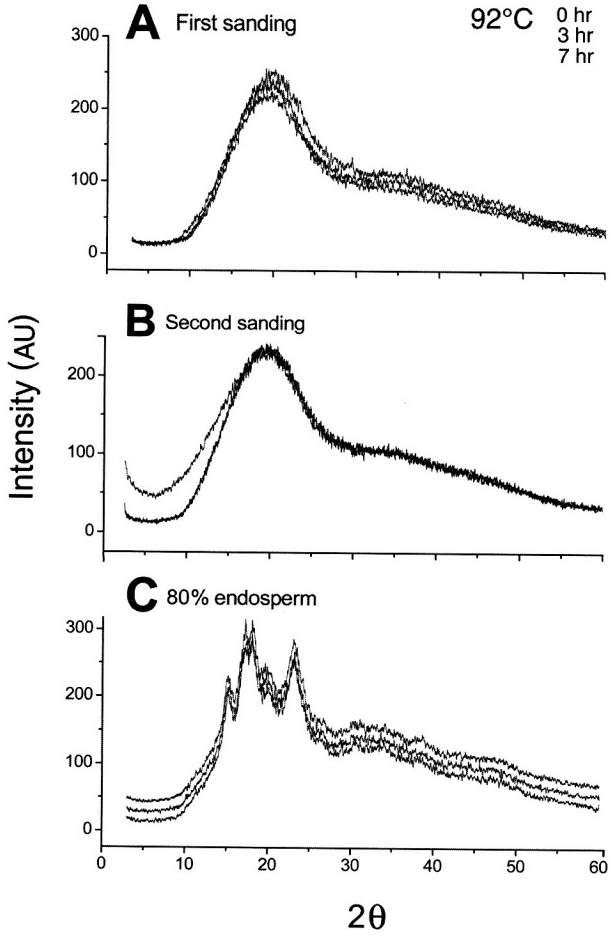

Fig. 8. A, X-ray diffraction patterns of the first sanding (outermost 10%); **B,** second sanding (subsequent 10%); and **C,** remaining 80% of the endosperm for samples steeped for 0, 3 and 7 hr and cooked at 92°C.

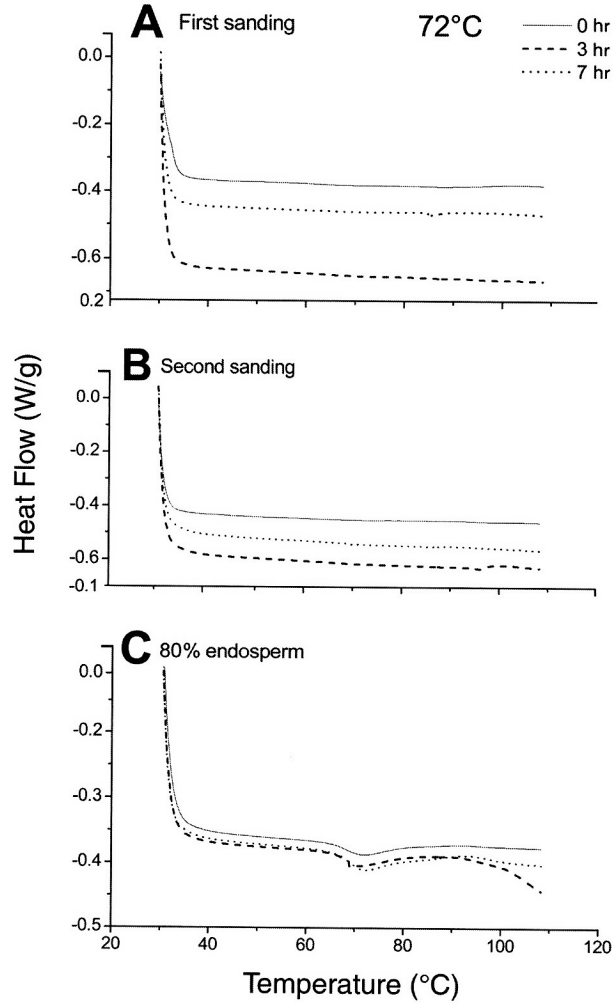

Fig. 9. DSC thermograms of samples steeped for 0, 3 and 7 hr and cooked at 72°C. **A,** first sanding (outermost 10%); **B,** second sanding (subsequent 10%); and **C,** remaining 80% of the endosperm.

The thermal treatment in water amorphizes the most external layers of the endosperm (≈20%). The nixtamalization process in the 72–92°C range produces the total gelatinization and melting of the starch of the most external layers of the endosperm and the partial disruption of the internal order in the most internal layers of the endosperm, according to the X-ray diffraction patterns and the DSC measurements in these structures. These results disagree with all previous reports (Gomez et al 1991, 1992), in which the crystalline starch structure was considered to be partially disrupted during the nixtamalization process by the analysis of the whole endosperm, while these studies were focused on different layers of this structure.

Finally, according to the X-ray diffraction and DSC results, a new definition of the nixtamalization process can be proposed: during the nixtamalization process, there is a total gelatinization of the starch granules of the most external layers and partial gelatinization of the most internal layers of the endosperm.

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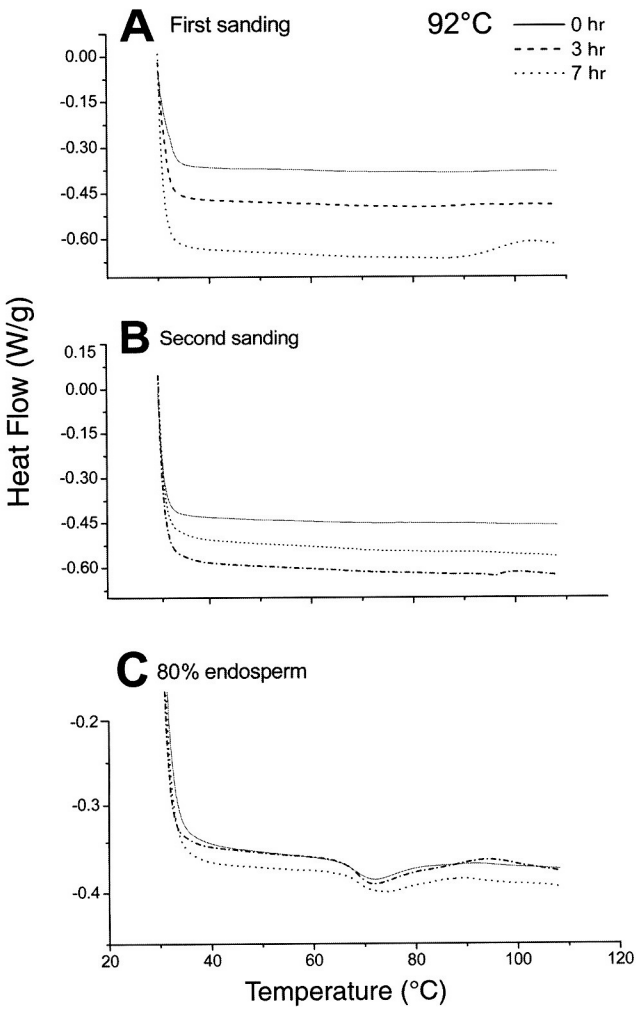

Fig. 10. DSC thermograms of samples steeped for 0, 3 and 7 hr and cooked at 92°C. **A,** first sanding (outermost 10%); **B,** second sanding (subsequent 10%); and **C,** remaining 80% of the endosperm.

LITERATURE CITED

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 08-01, 30-25, and 46-13. The Association: St. Paul, MN.

Almeida-Dominguez, H. D., Suhendro, E. L., and Rooney, L. W. 1997. Corn alkaline cooking properties related to grain characteristics and viscosity (RVA). J. Food Sci. 62:516-523.

AOAC International. 2000. Official Methods of Analysis of the Association of Official Analytical Chemists, 17th Ed. Methods 920.86 and 925.10. The Association: Gaithersburg, MD.

Atwell, W. A., Hood, L. F., Lineback, D. R., Varriano-Marston, E., and Zobel, H. F. 1988. The terminology and methodology associated with basic starch phenomena. Cereal Foods World. 33:306-311.

Bressani, R., Benavides, V., Acevedo, E., and Ortiz, M. A. 1990. Changes in selected nutrient content and in protein quality of common and quality protein maize during tortilla preparation. Cereal Chem. 67:515-518.

Dengate, H. N. 1984. Swelling, pasting, and gelling of wheat starch. Pages 49-82 in: Advances in Cereal Science and Technology, Vol. VI. Y. Pomeranz, ed. AACC International: St. Paul, MN.

Donovan, J. W., Lorenz, K., and Kulp, K. 1979. Differential scanning calorimetry of heat-moisture treated wheat and potato starches. Cereal Chem. 60:381-387.

Fernández-Muñoz, J. L., Rodriguez, M. E., Pless, R., Martínez-Flores, H. E., Leal, M., Martínez, J. L., and Baños, L. 2002. Changes in nixtamalized corn flour dependent on the post-cooking time. Cereal Chem. 79:162-166.

Fernández-Muñoz, J. L., Rojas-Molina, I., González-Dávalos, M. L., Leal, M., Valtierra, M. E., San Martín-Martínez, E., and Rodríguez, M. E. 2004. Study of calcium ion diffusion in components of maize kernels during traditional nixtamalization process. Cereal Chem. 81:65-69.

Gibbon, B. C., Wang, W., and Larkins, B. A. 2003. Altered starch structure is associated with endosperm modification in Quality Protein Maize. Proc. Nat. Acad. Sci. USA. 100:15329-15334.

Gomez, M. H., Waniska, R. D., and Rooney, L. W. 1991. Starch characterization of nixtamalized corn flour. Cereal Chem. 68:578-582.

Gomez, M. H., Lee, J. K., McDonough, C. M., Waniska, R. D., and Rooney, L. W. 1992. Corn starch changes during tortilla and tortilla chip processing. Cereal Chem. 69:275-279.

González, R., Reguera, E., Mendoza, L., Figueroa, J. M., and Sánchez-Sinencio, F. 2004. Physicochemical changes in the hull of corn grains during their alkaline cooking. J. Agric. Food Chem. 52:3831-3837.

Gutiérrez, E., Rojas-Molina, I., Pons-Hernández, J. L., Guzmán, H., Aguas-Angel, B., Arenas, J., Fernández, P., Palacios-Fonseca, A., Herrera, G., and Rodríguez, M. E. 2007. Study of calcium ion diffusion in nixtamalized quality protein maize as a function of the cooking temperature. Cereal Chem. 84:186-194.

Imberty, A., Chanzy, H., Perez, S., Burleon, A., and Tran, V. 1988, The double-helical nature of the crystalline part of A-starch. J. Mol. Biol. 201:365-378.

Jenkins, P. 1994. X-ray and neutron scattering studies on starch granule structure. PhD thesis. University of Cambridge: Cambridge, UK.

Krueger, B. R., Knutson, C. A., Inglett, G. E., and Walker, C. E. 1987. A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. J. Food Sci. 52:715-718.

Lelievre, J., and Mitchell, H. 1975. A pulsed NMR study of some aspects of starch gelatinization. Starch 27:113-115.

Martínez-Bustos, F., Morales, S. E., Chang, Y. K., Herrera-Gómez, A., Martínez, J. J., Baños, L., Rodríguez, M. E., and Flores, M. H. 1999. Effect of infrared baking on wheat flour tortilla characteristics. Cereal Chem. 76:491-495.

Mondragón, M., Bello-Perez, L. A., Agama., E., Melo, A., Betancur-Ancona, D., and Peña, J. L. 2004. Effect of nixtamalization on the modification of the crystalline structure of maize starch. Carbohydr. Polym. 55:411-418

Ramos, G., Pezet-Valdez, M., O'Connor-Sánchez, A., Placencia, C., and Pless, R. C. 2004. Hydration rates for various types of Mexican maize based on single-kernel measurements. Cereal Chem. 81:308-313.

Robles, R. R., Murray, E. D., and Paredes-Lopez, O. 1988. Physicochemical changes of maize starch during the lime-cooking treatment for tortilla making. Int. J. Food Sci. Technol. 23:1-98.

Rodríguez, M. E., Yáñez-Limón, J. M., Cruz-Orea, A., Alvarado-Gil, J. J., Zelaya-Ángel, O., Sánchez-Sinencio, F., Vargas, H., Figueroa, J. D. F., Martínez-Bustos, F., González-Hernández, J., Martínez-Montes, J., and

Miranda, L. C. M. 1995. The influence of the slake content on the processing conditions of cooking tortilla: Changes in thermal, structural rheological properties. Z. Lebensm Unsters Forsch 201:236-240.

Rodríguez, M. E., Yáñez-Limón, J. M., Alvarado, J. J., Vargas, H., Sánchez-Sinencio, F., Figueroa, F., Martínez, B. F., González-Hernández, J., Silva, M. D., and Miranda, L. C. M. 1996. Influence of the structural changes during alkaline cooking on the thermal, rheological, and dielectric properties of maize tortillas. Cereal Chem. 73:593-600.

Saas, J. E. 1977. Morphology. Pages 89-110 in: Corn Improvement, 3rd Ed. G. F. Sprague, ed. Am. Soc. Agron.: Madison, WI.

Sahai, D., and Jackson, D. S. 1999. Enthalpic transitions in native starch granules. Cereal Chem. 76:444-448.

Sahai, D., Mua, J. P., Surjewan, I., Buendia, M. O., Rowe, M., and Jackson, D. S. 1999. Assessing degree of cook during corn nixtamalization: Impact of processing variables. Cereal Chem. 76:850-854.

Sajilata, M., Singhal, S. R., and Kulkarni, P. R. 2006. Resistant starch—A Review. Comprehensive Reviews in Food Science and Food Safety. CRFSFS 5:1-17.

Sandhu, K. S., Singh, N., and Kaur, M. 2004. Characteristics of the different corn types and their grain fractions: Physicochemical, thermal, morphological, and rheological properties of starches. J. Food Eng. 64:119-127.

Sefa-Dedeh, S., Cornelius, B., Sakyi-Dawson, E., and Afoakwa, E. 2004. Effect of nixtamalization on the chemical and functional properties of maize. Food Chem. 86:317–324.

Serna-Saldivar, S. O., Gomez, M. H., and Rooney, L. W. 1990. Technology chemistry and nutritive value of alkaline-cooked corn products. Pages 243–295 in: Advances in Cereal Science and Technology. Y. Pomeranz, ed. AACC International: St. Paul, MN.

Wolf, M. J., Buzan, C. L., MacMasters, M. M., and Rist, C. E. 1952. Structure of the mature corn kernel. I. Gross, anatomy and structural relationships. Cereal Chem. 29:321-333.

Yglesias, R., Parkhurst, M. A., and Jackson, D. S. 2005. Development of laboratory techniques to mimic industrial-scale nixtamalization. Cereal Chem. 82:695-701.

Zallen, R. 1998. Amorphous morphology: The geometry and topology of disorder. Pages 33-51 in: The Physics of Amorphous Solids. John Wiley and Sons: New York.

Zobel, H. F., Young, S. N., and Rocca, L. A. 1988. Starch gelatinization: An X-ray diffraction study. Cereal Chem. 65:443-446.

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