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Effect of nixtamalization on the modification of the crystalline structure of maize starch

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Abstract

Endosperm of nixtamalized corn was analyzed using X-ray diffraction. Relative crystallinity changed with lime concentration and steeping. Diffractograms showed peaks corresponding to V-type crystalline structures, indicating formation of complexes during cooking and steeping. Diffraction patterns of the soluble fraction showed that complexed amylose can be leached out during solubilization. While diffraction patterns of the insoluble fraction suggested that some of the formed complexes remain in this fraction. During alkali steeping, release of amylose is strongly inhibited as indicated by the pronounced decrease in the starch–I_2 absorbance of the lime treated samples compared to the lime-free treated sample. This decrease is interpreted as evidence of starch cross-linking during the nixtamalization process. Differences in starch–I_2 absorbance and in X-ray diffraction patterns of the soluble fractions suggested that lime treatment could also modified formation of amylose complexes with lipids.

Keywords: Nixtamalization; Crystalline structures; Starch–lipid interaction; V polymorph

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1. Introduction

Nixtamalization is a process used to produce many Mexican foods, such as tamales and atole, including the most popular and most consumed of them all, tortillas (Paredes-López, Guzmán-Maldonado, & Serna-Saldivar, 2000). The demand for nixtamalized products has shown an important growth, not only in the United States but also in Asia and Europe (Almeida-Domínguez, Cepeda, & Rooney, 1996). The nixtamalization is carried out by cooking and steeping corn in lime solutions to obtain nixtamal (Paredes-López & Saharópulos, 1983).

During nixtamalization, maize components are affected by the alkaline cooking and steeping involved in the process. Starch is partially gelatinized, part of the cell wall components and proteins are solubilized, much of the germ tissue is retained and part of the lipids are saponified (Gomez, McDonough, Rooney, & Waniska, 1989; Serna-Saldivar, Gomez & Rooney, 1990; Robles, Murray, & Paredes-López, 1988). Also, calcium content increases and becomes highly available (Stylianopoulos, Serna-Saldivar, & Arteaga-MacKinney, 1991; Trejo-Gonzalez, Feria-Morales, & Wild-Altamirano, 1982).

Approximately 86–89% of the maize endosperm is starch, which consists of two major components: the linear amylose and the branched amylopectin. It is present in nature as semicrystalline granules. Crystallinity occurs within the ordered arrays of amylopectin (Oates, 1997) and according to X-ray diffraction analysis, native maize starch yields a pattern that corresponds to the A-type crystalline structure. The proposed model for the A structure is based upon six-fold, left-handed double helices packed parallel in a monoclinic unit cell (Gidley & Bociek, 1985).

It has been shown previously, that during alkali cooking, crystallinity of maize starch is reduced and tends to be recovered during steeping and cooling due to a recrystallization process (Gomez, Lee, McDonough,

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Waniska, & Rooney, 1992). Changes in crystallinity behavior of tortillas has also been reported as a function of lime concentration (Rodríguez et al., 1996). They found that crystallinity increased as lime cooking concentration increased at concentrations lower than 0.2%, but a reversal tendency was observed as lime concentration approached 1%. This behavior was attributed to calcium-induced cross-linking of starch.

Monoacyl lipids, present in native maize starch, can form complexes with the amylose during heat-moisture treatments, extrusion and gelatinization (Becker, Hill, & Mitchell, 2001; Biliaderis, 1980). These helical inclusion complexes typically display six residues per turn; the aliphatic part of the lipid is contained in the internal cavity and the polar group lies outside. The amylose–lipid complexes form V-type crystal structures which affect starch solubility, water absorption, and enzyme susceptibility as well as rheological and mechanical properties, depending on final crystallinity and crystal size (Galloway, Biliaderis, & Stanley, 1989; Le Bail et al., 1999). Nixtamalization could be carried out at several conditions leading to different degrees of starch gelatinization (Bryant & Hamaker, 1997). Thus, the presence of amylose–lipid complexes in nixtamalized products may be expected. Ocegueda (1999) has also suggested formation of amylose complexes with calcium ion-joined-lipids during nixtamalization. These complexes would contribute to obtain X-ray diffraction patterns characteristic of two polymorph forms, A and V, on nixtamalized samples. However, Toro-Vazquez & Gómez-Aldapa (2001) reported that extraction of free lipids did not affect the A-type diffraction pattern of isolated starches from nixtamalized maize, and concluded that amylose–lipid complexes are not developed during nixtamalization.

Considering contradictory previous data, in this study, we attempt to gain information to determine how the crystalline structure of starch changes due to the nixtamalization process, by evaluating directly endosperm samples of nixtamalized maize.

2. Experimental

2.1. Sample preparation

The samples were prepared as follows: one part of white dent corn and three parts of lime-water solutions (0, 0.2, and 1% w/v) were cooked at boiling temperature for 30 min and allowed to steep for 16 h. After steeping, the cooking solution was discarded and kernels were washed three times with distilled water. Samples were oven-dried at 50 °C for 36 h. Germ, tip cap and pericarp were removed before grinding the kernels to flour in a coffee mill. A second system of samples was prepared with the same procedure, but without steeping.

2.2. X-ray diffraction measurements

X-ray powder diffraction was performed with hydrated flour. Hydration of samples was done in a chamber maintained at 20 °C and 95% rh for 36 h. X-ray diffraction patterns were determined on a Bruker AXS D8 diffractometer (Madison, WI) operating a 40 kV, 35 mA with Cu K_α radiation ($\lambda = 0.154$ nm). Diffractograms were obtained from 5 to 30° (2θ) with a step size of 0.05° (2θ). All diffractograms were normalized at the same total area under the scattering curve over the measured Bragg angle range. Relative crystallinity was determined by the method of Wakelin, Virgin, and Crystal (1959), endosperm of raw corn was used as the crystalline standard. Amorphous standard was obtained according to Bogracheva, Wang, and Hedley (2001). A 5% aqueous suspension of corn starch was slowly heated to 100 °C with stirring, left at this condition for 1 h, then transferred to an oil-bath at 100 °C. The bath temperature was raised to 150 °C, followed by cooling to 100 °C. The samples were removed, cooled rapidly and lyophilized. The pattern of the amorphous starch was fitted to the to the pattern of nixtamalized samples (Murthy & Minor, 1990) and then subtracted, to obtain the pattern for the crystalline part of the starch.

For evaluation of the alkali treatment on amylose-lipid complex formation, dilute flour solutions (5% w/w) of the nixtamalized samples were heated to 90 °C, kept at this temperature for 30 min, then cooled rapidly to 20 °C. The soluble and insoluble fractions were separated, rapidly frozen and then freeze dried. X-ray diffraction patterns were also recorded and normalized for these samples.

2.3. Starch–I_2 absorbance analysis

Amylose–lipid complex formation, during nixtamalization, was also studied measuring starch–I_2 absorbance according to Ghiasi, Varriano-Marston, and Hoseney (1982), with some modifications. Dilute flour solutions (5%) were heated for 30 min to 95 °C, cooled rapidly in an ice bath, centrifugated and solubles separated. One milliliter of a I_2–KI dilute solution (2 mg/ml I_2 + 20 mg/ml KI) was added to 4 ml of the soluble fraction; 20 min later absorbance was measured.

3. Results and discussions

3.1. Crystallographic structures of nixtamalized starch

X-ray diffractograms of raw and nixtamalized samples not allowed to steep are shown in Fig. 1A. Main reflections of raw endosperm diffractogram (at Bragg angles 15.0, 17.0, 18.1, and 23.3° 2θ) are characteristic of the A-type starch structure typical of cereals (Zobel, 1988). Patterns for the crystalline part of the samples cooked at both lime concentrations (Fig. 1B) show a small reflection at 12.9°

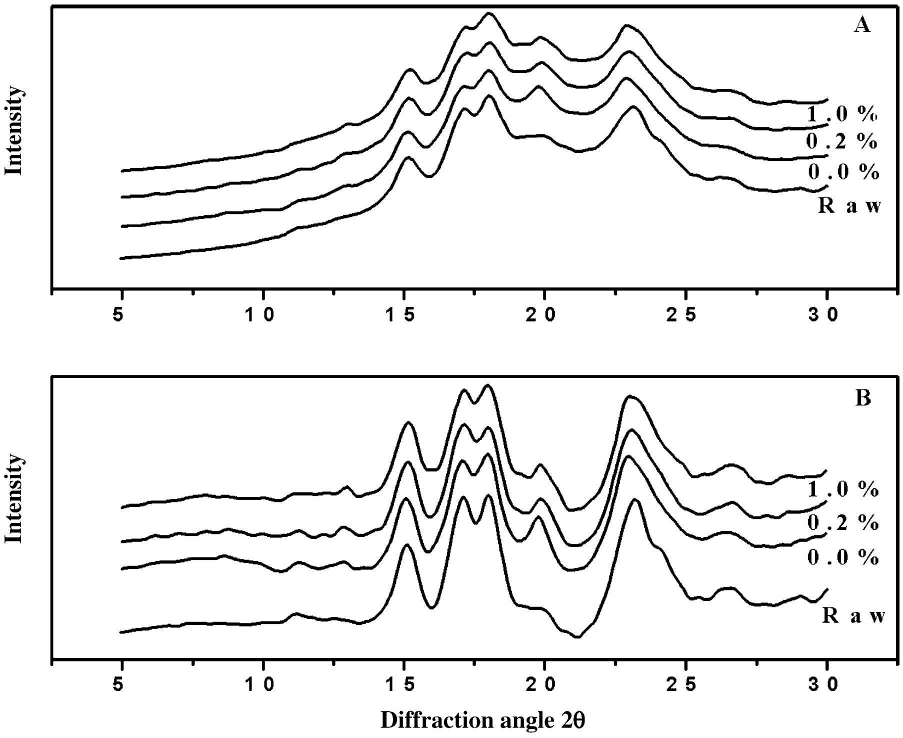

Fig. 1. X-ray diffraction patterns for nixtamalized samples not allowed to steep and for their corresponding crystalline parts (B), as a function of lime concentration (%, w/v).

and a larger reflection at 19.8° corresponding to the V-polymorph. Pattern of the lime-free cooked sample also presents reflections at the same angles. Studies using ^{13}C CP/MAS-NMR (Morrison, 1995) have proven the presence of amylose–lipid complexes in native granules of maize starch, but evidence of the coexistence of A- and V-type polymorphs by X-ray studies has only been found in starches containing more than 30% amylose (Zobel, 1988). However, it is known that during gelatinization of lipid containing-starches, amylose forms V-type helical crystalline complexes (Biliaderis, 1980). Considering that under nixtamalization conditions corn starch is partially gelatinized (Gomez et al., 1992), we assumed that the mentioned peaks correspond to complexes formed due to starch–lipid interactions.

Diffraction patterns of samples with steeping and their corresponding crystalline parts (Fig. 2A and B) are very similar to X-ray patterns of samples without steeping. Only a very low percent of starch was gelatinized during cooking as was demonstrated performing differential scanning calorimetry. The difference between gelatinization enthalpy for raw (7.8 J/g) and cooked samples (mean value ≈ 6.6 J/g) was not very large (data not shown). Therefore, structural reorganization which occurs due to retrogradation and annealing of starch during the nixtamalization process (Gomez et al., 1992) is negligible at these conditions.

Relative crystallinities of the nixtamalized samples are given in Table 1. The crystallinity of the samples without steeping tend to increase with increasing lime concentration. For the samples with steeping, crystallinity increases at 0.2% lime concentration, however, it tends to decrease to a similar value to that of the lime-free sample at 1.0% lime concentration. Rodríguez et al. (1996) reported a similar crystallinity behavior for tortillas, as mentioned before, which was attributed to cross-links formation leading to more rigid network at low lime concentrations. The crystallinity behavior of samples without steeping seems to indicate that during cooking only a small amount of cross-links are formed at low lime concentration; therefore, granule rigidity is not increased.

3.2. Effect of lime-treatment on V-type crystal structure

Diffraction patterns of the soluble starches of nixtamalized maize at 0, 0.2, and 1% lime concentration with or without steeping (Figs. 3A and 4A) present a peak at Bragg angle 19. 8° and a shoulder at 12.9° 2θ, which are associated with the V polymorph. The X-ray patterns for the crystalline part of these samples corroborate the presence of the characteristic peak of the V polymorph at 12.9° 2θ (Figs. 3B and 4B). Complexed amylose with lipids does not leach out easily from the granules when heated in water (Becker et al., 2001). However, other investigators (Ghiasi et al., 1982) reported the release of surfactant–amylose complexes into the aqueous phase at temperatures higher than 85 °C. Also, Colonna, Buléon, and Mercier (1987) have reported transformation of amylose–lipid complexes into an extractable form during heat-moisture treatment of cereal

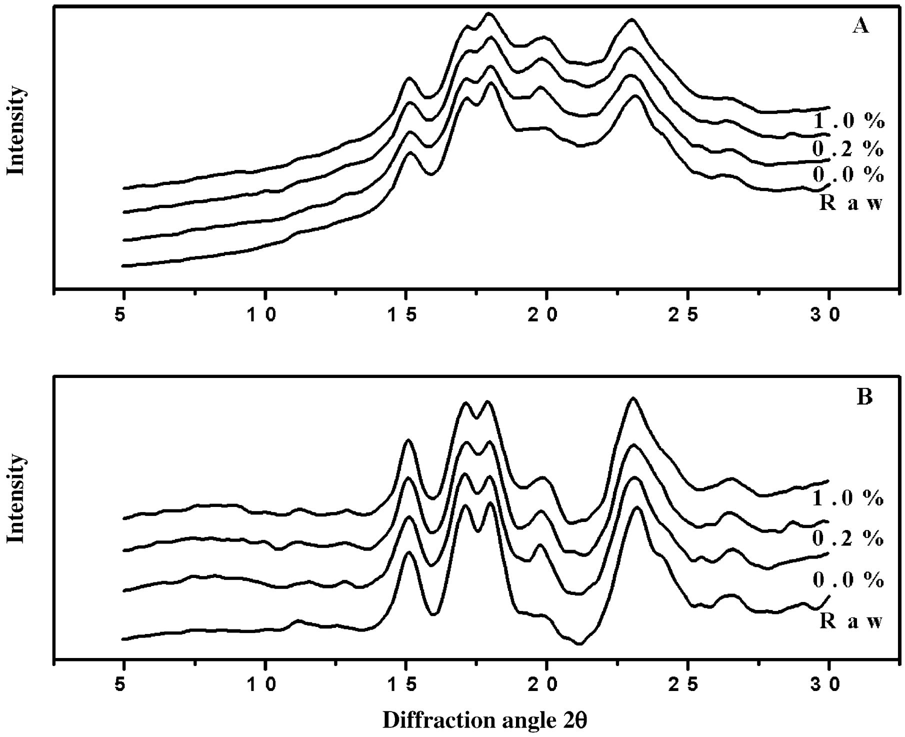

Fig. 2. X-ray diffraction pattern for nixtamalized samples allowed to steep (A) and for their corresponding crystalline parts (B), as a function of lime concentration (%, w/v).

starches. These reports could help to explain the V-type reflections in the diffraction diagram of the soluble starches. Considering that our samples were air-dried at 50 °C for 36 h before grinding, and soluble fraction obtained by heating the flour suspensions at 100 °C, it may be assumed that some amylose complexes could have been released during the leaching process. Retrogradation of leached amylose also occurs upon cooling the soluble fraction. Gidley (1989) has postulated that amylose association includes aggregation of double helices to form B-type structures. The reflection at 17° 2θ shown in the diffractograms of the solubles indicates the existence of the B-type polymorph which would correspond to the associated amylose.

X-ray diffraction diagrams of the insoluble starches at all lime concentrations used are shown in Figs. 5A and 6A. The small reflection at Bragg angle 19.8° 2θ, indicates the possibility that V-type structures be formed in these fractions. Since scattering register is weak, it might be assumed that V-crystals are very small and ordering is relatively low. This suggestion is supported by the broad reflection shown to the left of 20° 2θ, in the patterns for the crystalline part of the insoluble fractions (Figs. 5B and 6B) which present the semblance of a peak at 12.9° 2θ. As mentioned above, diffraction diagrams for the crystalline part of cooked samples with or without steeping also present V-type reflections (Figs. 1B and 2B), as well as the patterns corresponding to their soluble fractions (Figs. 3B and 4B). As previously discussed, when starch is cooked in lime water, calcium may form cross-links between the starch chains (Bryant & Hamaker, 1997; Rodríguez et al., 1996) reducing amylose and amylopectin solubility (Jackson, Choto-Owen, Waniska, & Rooney, 1988). Then, it is possible that a small amount of the formed complexes remains in the insoluble sediment. Most of amylose–lipid complexes would be leached out from starch granules when the flour of these samples is heated in water, yielding stronger diffraction peaks of the V type in the soluble fraction.

Table 1
Relative crystallinity of nixtamalized samples

Lime concentration (%)	Relative crystallinity (%)	
	Without steeping	With steeping
0.0	89.4	89.6
0.2	89.3	97.4
1.0	97.4	88.2

3.3. Changes in starch–I_2 absorbance

Amylose can form helical complexes, not only with polar lipids, but also with some alcohols and iodine (Thomas & Atwell, 1999). The complex of amylose with iodine produces a blue color; its intensity could be used to determine the level of amylose in the solubles of a starch

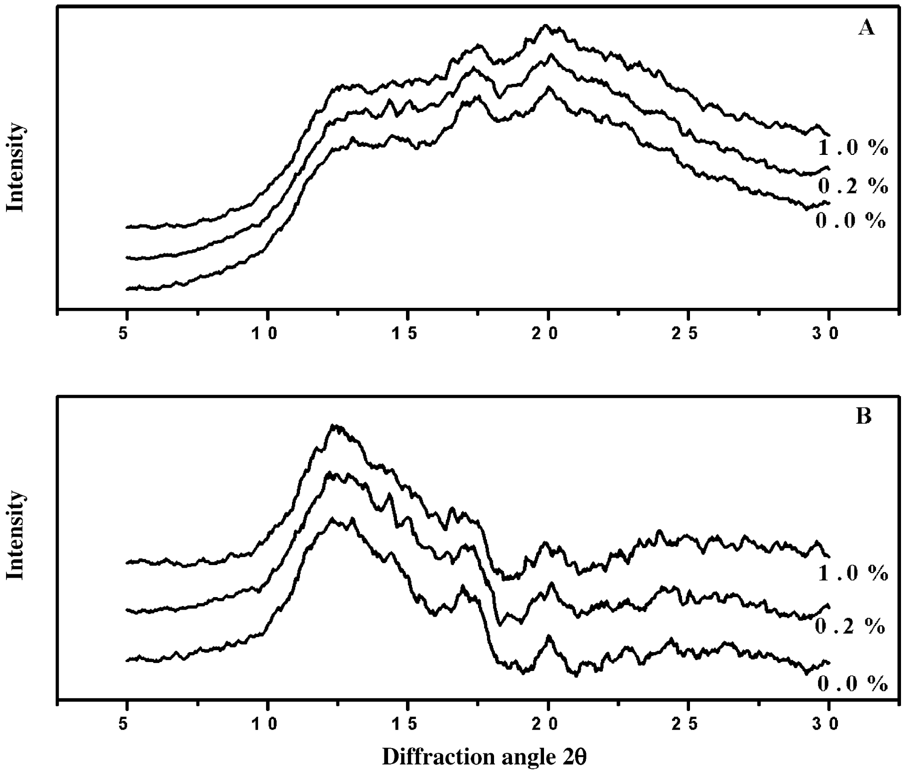

Fig. 3. X-ray diffraction patterns of the solubles of nixtamalized samples not allowed to steep (A) and for their corresponding crystalline parts (B), as a function of lime concentration (%, w/v).

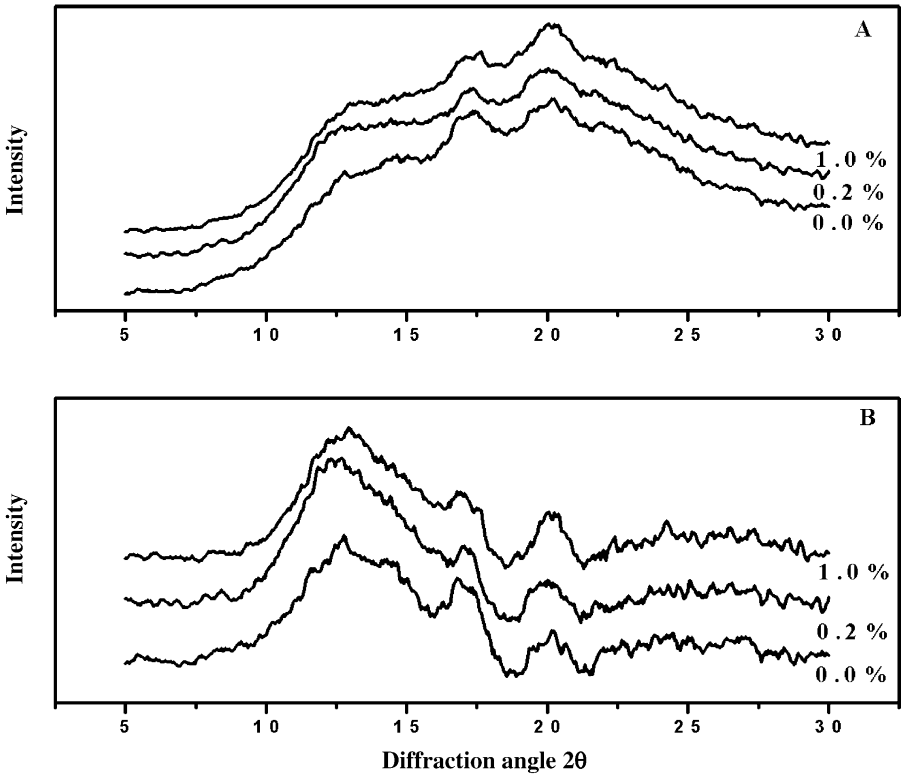

Fig. 4. X-ray diffraction patterns of the solubles of nixtamalized samples allowed to steep (A) and for their corresponding crystalline parts (B), as a function of lime concentration (%, w/v).

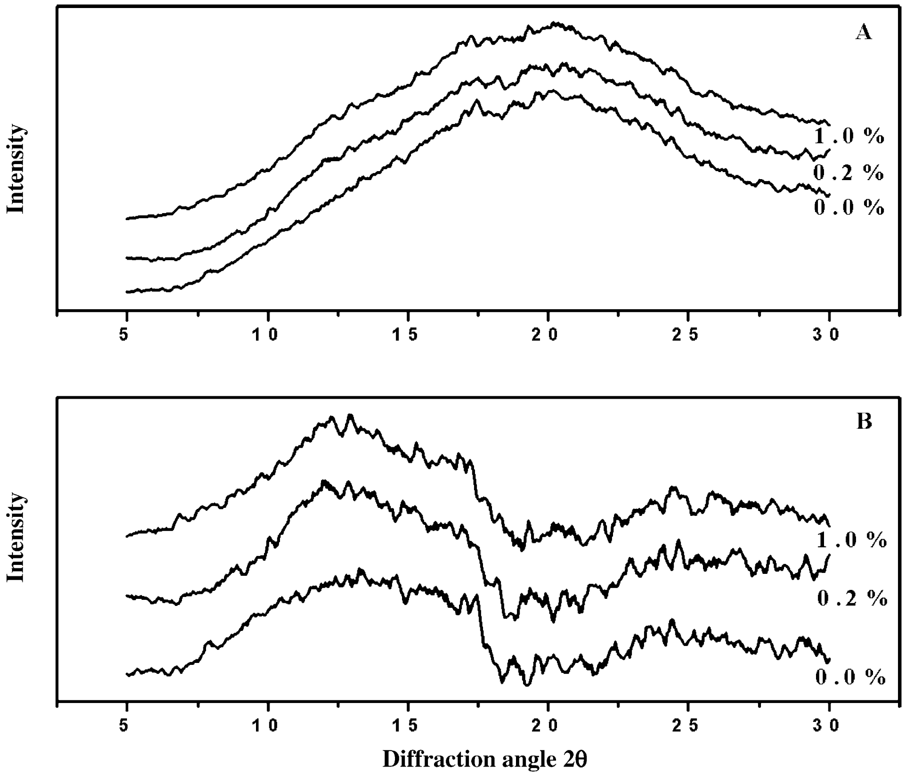

Fig. 5. X-ray diffraction patterns of the insoluble residues of samples not allowed to steep (A) and for their corresponding crystalline parts (B), as a function of lime concentration (%, w/v).

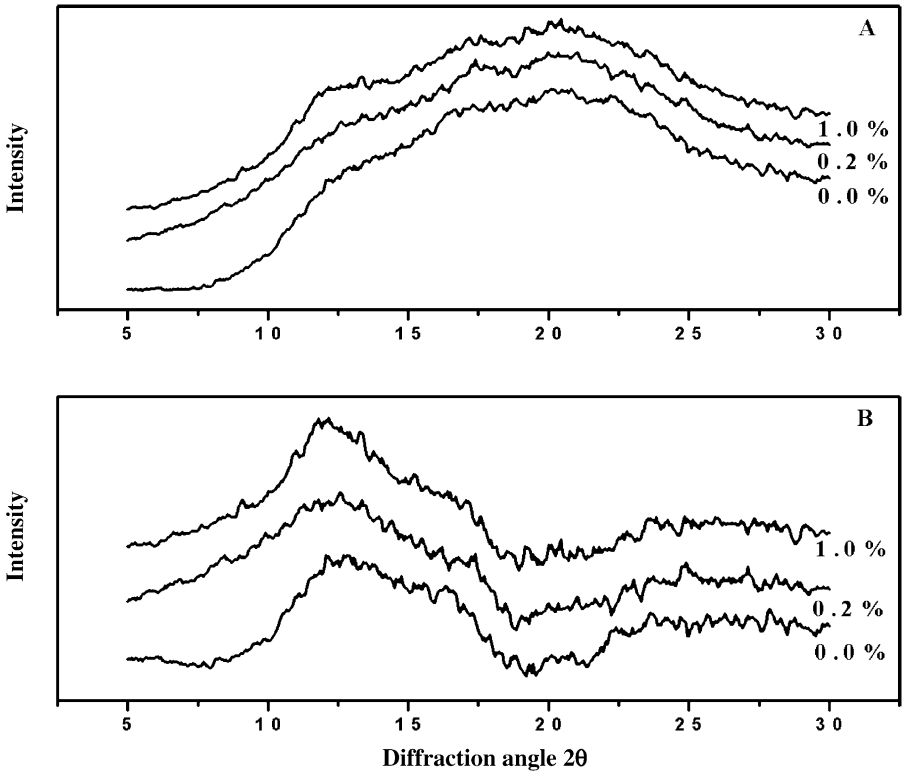

Fig. 6. X-ray diffraction patterns of the insoluble residues of samples allowed to steep (A) and for their corresponding crystalline parts (B), as a function of lime concentration (%, w/v).

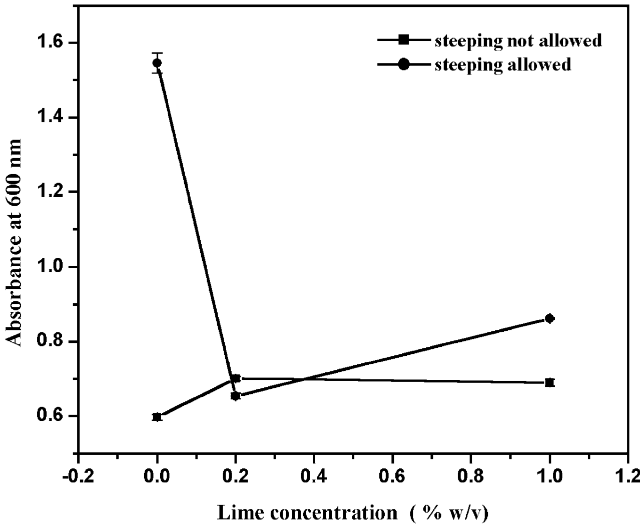

Fig. 7. Effect of lime on iodine absorption of nixtamalized samples.

solution (Morrison & Laignelet, 1983). Our results on absorbance of samples not allowed to steep, with or without lime, are quite similar (Fig. 7). However, when lime-free cooked sample is allowed to steep a significant change is observed: the level of released amylose in the solubles that can complex with iodine is strongly increased compared to samples steeped in lime solutions. The calcium cross-links formed during the alkali steeping could decrease release of starch chains ready to complex with iodine. Amylose–lipid-complexes also affect starch solubilization (their presence in samples with steeping was demonstrated by their X-ray diffraction patterns, Fig. 2) therefore, absorbance behavior could be attributed to both phenomena. Since cross-linking could affect, in turn, the amount of starch chains ready to complex with lipids it is postulated that formation these complexes may be affected by the Ca–starch interactions. Visual examination of the X-ray diffraction patterns for the soluble fraction of nixtamalized samples without and with steeping (Figs. 3 and 4) revealed small differences in the intensity of the peak at Bragg angles 12.9° and 19.8° (2θ) which could be taken as indicators of amylose–lipid crystallites being modified. The differences in relative crystallinities (Table 1) also seem to support this suggestion. X-ray analysis on starches showing both B and V polymorphs, suggested that cross-linking affect the development of crystalline structures (Le Bail, Morin, & Marchessault, 1999). The change in solubility of amylose or in the content of amylose–lipid complexes will affect rheological properties of masa and tortillas, since it is known that helical amylose–lipid complexes induce changes in viscosity of cereal starches (Nierle & El Baya, 1990; Kaur & Singh, 2000) and also that leached amylose contributes to increased viscosity even when granule swelling is not modified (Tester & Morrison, 1990).

4. Conclusions

During nixtamalization amylose–lipid complexes are developed in the starch. These V-amylose complexes can be leached out during starch solubilization. Weak reflections in the X-ray diffraction patterns of the insoluble part from nixtamalized samples suggest that V-structures are also present in the insoluble residues. Absorbance results seem to confirm formation of starch cross-linking due to alkali treatment. Apparently, alkali steeping also affects formation of amylose–lipid complexes, however further investigation is needed to support this hypothesis.

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