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Research note

Method to estimate crystallinity in *nixtamalized* corn pericarp from sequential extractions and X-ray diffraction

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This Research Note presents a method to estimate the crystallinity in *nixtamalized* corn pericarp. A sequential extraction procedure that involves the separation of the water soluble, alkali soluble and lignin fractions and the precipitation of the supernatants was performed to separate the crystalline and amorphous components in the crude pericarp. From the x-ray diffraction measurements of the obtained fractions, a method to fit the crude pericarp diffractogram and then calculate the crystallinity was developed. The evolution of the crystallinity during a typical *nixtamalization* process was assessed and it was determined that the *nixtamalization* process extracts mainly the amorphous component identified as Hemi C, while the one assigned to Hemi B is only partially removed. It was demonstrated that the *nixtamalization* does not affect substantially the crystalline cellulose in the pericarp. Though it is time consuming, the method proved to be more reliable in comparison with the parabolic fitting of the amorphous-to-crystalline regions in the X-ray diffractograms usually reported in the literature, and it could be easily implemented for other natural materials.

Crystallinity is a fundamental property that relates the structure of polymeric materials within its preparation conditions and potential applications and is defined as a ratio between the crystalline material and its total weight (Alexander, 1985). The crystallinity has been related with functional properties such as recyclability (Tschirner et al., 2007), tensile properties (Silvério et al., 2013), feasibility of enzyme production onto cellulosic substrates (Brijwani and Vadlani, 2011) and stability upon thermal degradation (Miranda et al., 2013) amongst others. For crystallinity determination, a number of techniques can be used such as Nuclear Magnetic Resonance (Gonzalez et al., 2004), Differential Scanning Calorimetry (Castro-Lopez et al., 2014), Infrared Spectroscopy (Siroky et al., 2014) and X-ray diffraction (XRD) (Segal, 1959, Alexander, 1985), with respective comparative advantages, although only XRD provides direct information on the crystalline and amorphous contents of a material. In a semicrystalline polymer, the intensity of the upcoming x-ray radiation from the analyzed material does not depend on its ordered or disordered state. Therefore, the simplest method to determine its crystallinity by XRD is to separate the amorphous and crystalline contributions to the diffractogram by drawing a demarcation line connecting the minima of the crystalline peaks and calculating the crystallinity χ_c as the ratio between A_c and the total diffractogram area (A_t) (Alexander, 1985).

In cellulose materials, Segal (Segal, 1959) proposed a crystallinity index (CrI) that considers the diffracted intensities of the (002) plane of cellulose at 2theta = 22° and the intensity of diffraction in the same units at 2theta = 18°. I_{002} represents both crystalline and amorphous regions, while I_{AM} represents only the amorphous part. In a recent work, a more elaborate procedure to fit the X-ray diffractograms of cellulose material using several reflections has been proposed (Brijwani and Vadlani, 2011):

$$Xcr(\%) = (I_{002} + I_{021})/(I_{101} + I_{101} + I_{002} + I_{021} + I_{040}) \times 100 \quad (1)$$

where I followed by a subscript represents the integrated intensity of the particular Bragg plane. Crystallinity, therefore, represents the fraction of α-cellulose represented by planes (002) and (021) present in a particular sample.

However, in biological materials such as seed hulls, the drawing of this rather arbitrary demarcation line or the use of a single or several peak intensities has the drawback to partially disregard the inherent complexity of its multicomponent nature, and usually overestimate the calculated crystallinity, that could be an issue

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particularly when the crystallinity evolution is intended to be followed during processing.

Corn pericarp is roughly composed of 16% cellulose, 1% hemicellulose A (Hemi A from hereafter), 57% hemicellulose B (Hemi B), 14% hemicellulose C (Hemi C) and 2% lignin and minor amounts of fat, protein and minerals (Sugawara et al., 1994).

The polysaccharides extracted from corn pericarp during alkaline lime cooking – named *nixtamalization* in Mexico-consist in acidic heteroxylanes with polyelectrolyte character and a highly branched and interlinked structure where the cellulose microfibrils are embedded (Saulnier et al., 1993, Saulnier and Thibault, 1999). This structure confers to corn pericarp an increased resistance to degradation with respect to oat and wheat bran (Mongeau et al., 1991).

Corn pericarp influences some of the properties of *nixtamalized* products such as flavor, mechanical resistance, flexibility and rollability (Paredes-Lopez and Saharopulos Paredes, 1983) and contents of dietary fiber and calcium (FAO, 1992). Moreover, from *nixtamalized* corn pericarp, profitable byproducts could be obtained: food additives from the pericarp heteroxylans (Martínez-López et al., 2013); vanillic acid and guaiacol from the ferulic acids (Hosny and Rosazza, 1997); ethanol and hemicelluloses (Gaspar et al., 2007) as well as cellulose and dietary fiber (Zambrano-Zaragoza et al., 2013), amongst other uses.

In a recent work (Caballero-Briones et al., 2014) we observed that upon *nixtamalization*, the amorphous fraction of corn pericarp dissolves and cellulose fibers swell while calcium contents increases 20 times. To determine the crystallinity of the corn pericarp and later assess the crystallinity evolution of the *nixtamalized* samples, an extraction procedure depicted in Fig. 1 and described in the Supplementary Information was followed and the recovered fractions (Table S1, Supplementary Information) were measured by X-ray diffraction (Fig. 2a) and compared with micronized and microcrystalline celluloses (Fig. 2b).

Peaks at $2\theta = 23^\circ$, corresponding to the (002) reflection, at $2\theta \approx 21^\circ$ related with the (021) plane and peaks at $2\theta \approx 15^\circ$ and $2\theta \approx 16.5^\circ$ that correspond to the superposition of the (−101), non-assigned (na), (−111) and (101) reflections of cellulose I are observed in the diffractograms of the pericarp holocellulose sample (HC) and in those of EC (micronized cellulose) and CM (microcrystalline cellulose) (ICDD, 1997). As the extraction proceeds from PC (raw pericarp) to MIN (residue insoluble in NaOH) sample, it can be observed that the A_1 shoulder reaches its minimum intensity and the A_2 and A_3 zones present an important diminishing from MIN with respect to MIH (water insoluble residue) while the peaks corresponding to the (002) and (−111) cellulose planes became defined. The final extraction step, i.e. the MIN to HC sample only causes a slight decrease in the A_2 intensity. It is known that Hemi A and Hemi B in corn pericarp can be dissolved with a 1% NaOH extraction at room temperature, while Hemi C can only be isolated with a 17.5% NaOH extraction at room temperature (Saulnier et al., 1995; Sugawara et al., 1994). Donner and Hicks (1997) had shown that a mixture of Hemi A and Hemi B is obtained by extracting the corn pericarp during 1 h in boiling NaOH and/or $Ca(OH)_2$; from the mixture Hemi A and Hemi B can be separated but with a poor yield. It has been stated that Hemi B is more difficult to extract than Hemi A in similar conditions, probably because of a lignin or protein interlinking (Hespell, 1998; Saulnier et al., 1995; Sugawara et al., 1994).

Fig. 3a presents the X-ray diffractogram of the HMB sample (neutralized, EtOH precipitated supernatant after the NaOH extraction, Fig. 1). The diffractogram is a unique wide halo centered at $2\theta = 19^\circ$ corresponding to the A_2 center, very similar to that obtained by George et al. (1999) in amorphous pectin. The halo was fitted to a Lorentzian curve and the diffraction parameters *i.e.* position and width, were obtained. From hereafter, the fitted curve will be named L_2 (Fig. 3b). The L_2 curve intensity was linearly operated to match its intensity to that at $2\theta = 19^\circ$ in HC to then obtain the HC−L_2 curve, that corresponds to the crystalline material plus the

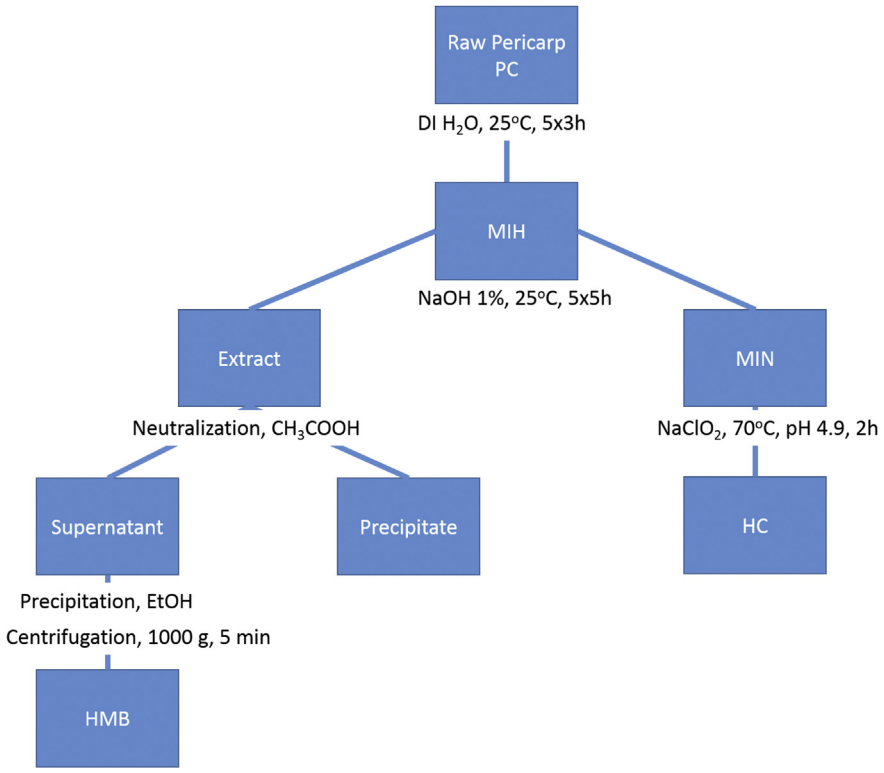

Fig. 1. Scheme of the sequential extraction procedure performed to obtain the structural fractions of the corn pericarp.